



(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention
of the grant of the patent:
02.06.2004 Bulletin 2004/23

(51) Int Cl.7: **C07F 15/00, C12Q 1/00,
G01N 27/49**

(21) Application number: **00978635.1**

(86) International application number:
PCT/US2000/031268

(22) Date of filing: **14.11.2000**

(87) International publication number:
WO 2001/036430 (25.05.2001 Gazette 2001/21)

(54) **TRANSITION METAL COMPLEXES WITH BIDENTATE LIGAND HAVING AN IMIDAZOLE RING**
ÜBERGANGSMETALL-KOMPLEXVERBINDUNGEN MIT EINER BIDENTATEN LIGANDE MIT
EINEM IMIDAZOL-RING
COMPLEXES DE METAUX DE TRANSITION A LIGAND BIDENTE PRESENTANT UN CYCLE
IMIDAZOLE

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE TR

(30) Priority: **15.11.1999 US 165565 P**

(43) Date of publication of application:
14.08.2002 Bulletin 2002/33

(73) Proprietor: **Therasense, Inc.**
Alameda, CA 94502 (US)

(72) Inventors:
• **MAO, Fei**
Fremont, CA 94555 (US)

• **HELLER, Adam**
Austin, TX 78759 (US)

(74) Representative: **Andrae, Steffen, Dr. et al**
Andrae Flach Haug
Balanstrasse 55
81541 München (DE)

(56) References cited:
WO-A-98/35225

• **OHARA, T. J. ET AL: "Glucose electrodes based**
on crosslinked [Os(bpy)2C1]+/2+ complexed
poly(1-vinylimidazole) films" POLYM. MATER.
SCI. ENG., vol. 70, 1993, pages 182-183,
XP000981691

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description**Field of the Invention**

- 5 [0001] This invention relates to transition metal complexes with at least one bidentate ligand containing at least one imidazole ring. In addition, the invention relates to the use of the transition metal complexes as redox mediators.

Background of the Invention

- 10 [0002] Enzyme based electrochemical sensors are widely used in the detection of analytes in clinical, environmental, agricultural and biotechnological applications. Analytes that can be measured in clinical assays of fluids of the human body include, for example, glucose, lactate, cholesterol, bilirubin and amino acids. Levels of these analytes in biological fluids, such as blood, are important for the diagnosis and the monitoring of diseases.

- 15 [0003] Electrochemical assays are typically performed in cells with two or three electrodes, including at least one measuring or working electrode and one reference electrode. In three electrode systems, the third electrode is a counter-electrode. In two electrode systems, the reference electrode also serves as the counter-electrode. The electrodes are connected through a circuit, such as a potentiostat. The measuring or working electrode is a non-corroding carbon or metal conductor. Upon passage of a current through the working electrode, a redox enzyme is electrooxidized or electroreduced. The enzyme is specific to the analyte to be detected, or to a product of the analyte. The turnover rate of the enzyme is typically related (preferably, but not necessarily, linearly) to the concentration of the analyte itself, or to its product, in the test solution.

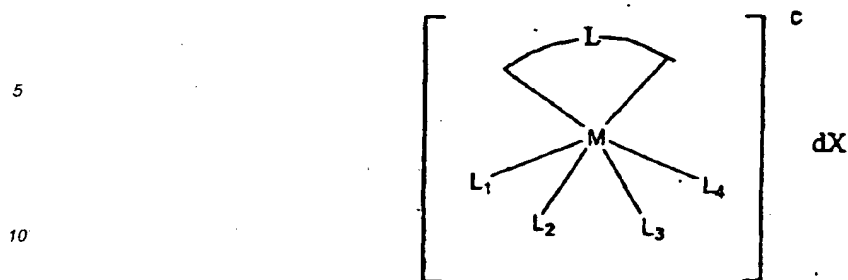
- 20 [0004] The electrooxidation or electroreduction of the enzyme is often facilitated by the presence of a redox mediator in the solution or on the electrode. The redox mediator assists in the electrical communication between the working electrode and the enzyme. The redox mediator can be dissolved in the fluid to be analyzed, which is in electrolytic contact with the electrodes, or can be applied within a coating on the working electrode in electrolytic contact with the analyzed solution. The coating is preferably not soluble in water, though it may swell in water. Useful devices can be made, for example, by coating an electrode with a film that includes a redox mediator and an enzyme where the enzyme is catalytically specific to the desired analyte, or its product. In contrast to a coated redox mediator, a diffusional redox mediator, which can be soluble or insoluble in water, functions by shuttling electrons between, for example, the enzyme and the electrode. In any case, when the substrate of the enzyme is electrooxidized, the redox mediator transports electrons from the substrate-reduced enzyme to the electrode; when the substrate is electroreduced, the redox mediator transports electrons from the electrode to the substrate-oxidized enzyme.

- 25 [0005] Recent enzyme based electrochemical sensors have employed a number of different redox mediators such as monomeric ferrocenes, quinoid-compounds including quinines (e.g., benzoquinones), nickel cyclamates, and ruthenium amines. For the most part, these redox mediators have one or more of the following limitations: the solubility of the redox mediators in the test solutions is low, their chemical, light, thermal, or pH stability is poor, or they do not exchange electrons rapidly enough with the enzyme or the electrode or both. Additionally, the redox potentials of many of these reported redox mediators are so oxidizing that at the potential where the reduced mediator is electrooxidized on the electrode, solution components other than the analyte are also electrooxidized; in other cases they are so reducing that solution components, such as, for example, dissolved oxygen are also rapidly electroreduced. As a result, the sensor utilizing the mediator is not sufficiently specific.

Summary of the Invention

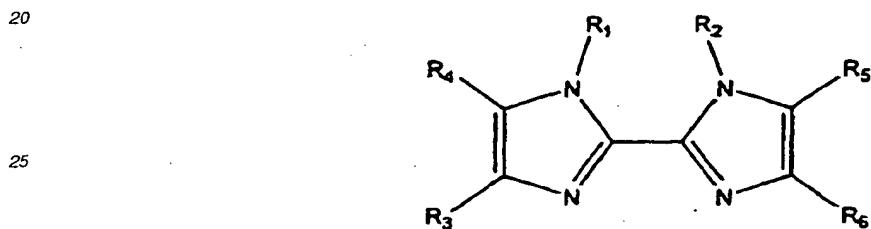
- 45 [0006] The present invention is directed to novel transition metal complexes. The present invention is also directed to the use of the complexes as redox mediators. The preferred redox mediators typically exchange electrons rapidly with enzymes and electrodes, are stable, and have a redox potential that is tailored for the electrooxidation of analytes, exemplified by glucose.

- 50 [0007] One embodiment of the invention is a transition metal complex having the formula:

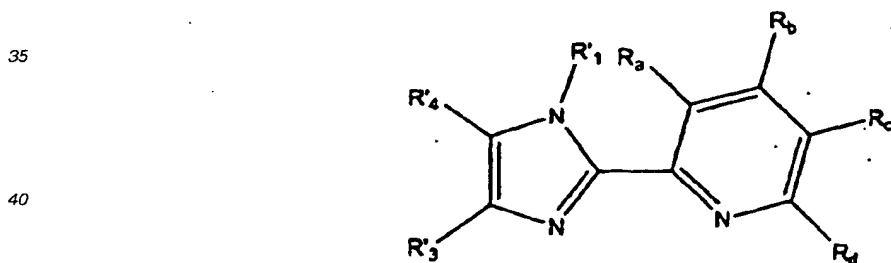


wherein

M is cobalt, iron, ruthenium, osmium, or vanadium;
L is selected from the group consisting of



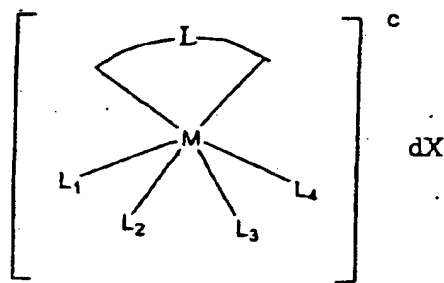
and



45

R_1 , R_2 , and R'_1 are independently substituted or unsubstituted alkyl, alkenyl, or aryl groups;
 R_3 , R_4 , R_5 , R_6 , R'_3 , R'_4 , R'_a , R'_b , R'_c , and R'_d are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂,
 50 -SH, -OH, -NH₂, or substituted or unsubstituted alkoxy, carbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkoxy,
 alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyami-
 no, alkylthio, alkenyl, aryl, or alkyl;
 c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge;
 X represents at least one counter ion;
 d is an integer from 1 to 5 representing the number of counter ions, X; and
 55 L_1 , L_2 , L_3 and L_4 are ligands.

[0008] Another embodiment concerns the use of a transition metal complex having the formula



wherein

M is iron, cobalt, ruthenium, osmium, or vanadium;

L is a bidentate ligand comprising at least one imidazole ring,

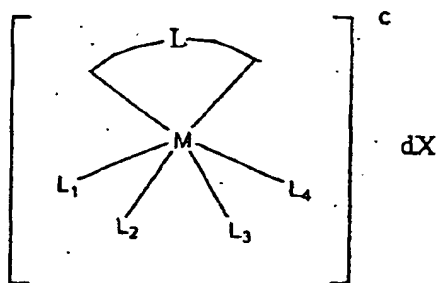
c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge;

X represents at least one counter ion;

d is an integer from 1 to 5 representing the number of counter ions, X; and

L₁, L₂, L₃ and L₄ are ligands, as redox mediator.

[0009] Yet another embodiment is a polymer comprising a polymeric backbone and a transition metal complex having the formula



wherein

M is iron, cobalt, ruthenium, osmium, or vanadium;

L is a bidentate ligand comprising at least one imidazole ring;

c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge;

X represents at least one counter ion;

d is an integer from 1 to 5 representing the number of counter ions, X; and

L₁, L₂, L₃ and L₄ are ligands.

wherein at least one of L, L₁, L₂, L₃ and L₄ is coupled to the polymeric backbone.

[0010] Yet another embodiment is a sensor comprising a working electrode, a counter electrode and a transition metal complex as it is defined above, which is used as redox mediator, disposed proximate to the working electrode.

Detailed Description

[0011] When used herein, the following definitions define the stated term:

[0012] The term "alkyl" includes linear or branched, saturated aliphatic hydrocarbons. Examples of alkyl groups include methyl, ethyl, n-propyl isopropyl, n-butyl, tert-butyl and the like. Unless otherwise noted, the term "alkyl" includes

both alkyl and cycloalkyl groups.

[0013] The term "alkoxy" describes an alkyl group joined to the remainder of the structure by an oxygen atom. Examples of alkoxy groups include methoxy, ethoxy, n-propoxy, isopropoxy, butoxy, *tert*-butoxy, and the like. In addition, unless otherwise noted, the term 'alkoxy' includes both alkoxy and cycloalkoxy groups.

[0014] The term "alkenyl" describes an unsaturated, linear or branched aliphatic hydrocarbon having at least one carbon-carbon double bond. Examples of alkenyl groups include ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-methyl-1-propenyl, and the like.

[0015] A "reactive group" is a functional group of a molecule that is capable of reacting with another compound to couple at least a portion of that other compound to the molecule. Reactive groups include carboxy, activated ester, sulfonyl halide, sulfonate ester, isocyanate, isothiocyanate, epoxide, aziridine, halide, aldehyde, ketone, amine, acrylamide, thiol, acyl azide, acyl halide, hydrazine, hydroxylamine, alkyl halide, imidazole, pyridine, phenol, alkyl sulfonate, halotriazine, imido ester, maleimide, hydrazide, hydroxy, and photo-reactive azido aryl groups. Activated esters, as understood in the art, generally include esters of succinimidyl, benzotriazolyl, or aryl substituted by electron-withdrawing groups such as sulfo, nitro, cyano, or halo groups; or carboxylic acids activated by carbodiimides.

[0016] A "substituted" functional group (e.g., substituted alkyl, alkenyl, or alkoxy group) includes at least one substituent selected from the following: halogen, alkoxy, mercapto, aryl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, -NH₂, alkylamino, dialkylamino, trialkylammonium, alkanoylamino, arylcarboxamido, hydrazino, alkylthio, alkenyl, and reactive groups.

[0017] A "biological fluid" is any body fluid or body fluid derivative in which the analyte can be measured, for example, blood, interstitial fluid, plasma, dermal fluid, sweat, and tears.

[0018] An "electrochemical sensor" is a device configured to detect the presence of or measure the concentration or amount of an analyte in a sample via electrochemical oxidation or reduction reactions. These reactions typically can be transduced to an electrical signal that can be correlated to an amount or concentration of analyte.

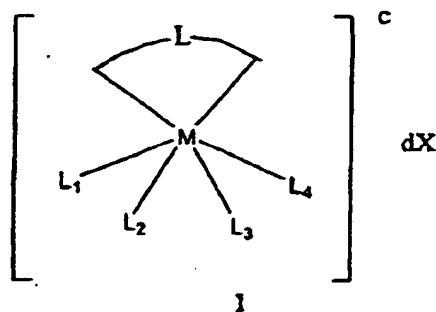
[0019] A "redox mediator" is an electron transfer agent for carrying electrons between an analyte or an analyte-reduced or analyte-oxidized enzyme and an electrode, either directly, or via one or more additional electron transfer agents.

[0020] "Electrolysis" is the electrooxidation or electroreduction of a compound either directly at an electrode or via one or more electron transfer agents (e.g., redox mediators or enzymes).

[0021] The term "reference electrode" includes both a) reference electrodes and b) reference electrodes that also function as counter electrodes (i.e., counter/reference electrodes), unless otherwise indicated.

[0022] The term "counter electrode" includes both a) counter electrodes and b) counter electrodes that also function as reference electrodes (i.e., counter/reference electrodes), unless otherwise indicated.

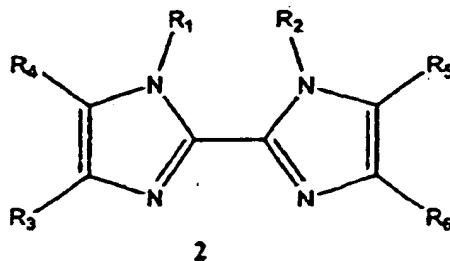
[0023] Generally, the present invention relates to transition metal complexes of iron, cobalt, ruthenium, osmium, and vanadium having at least one bidentate ligand containing an imidazole ring. The invention also relates to the use of the transition metal complexes as redox mediators. In at least some instances, the transition metal complexes have one or more of the following characteristics: redox potentials in a particular range, the ability to exchange electrons rapidly with electrodes, the ability to rapidly transfer electrons to or rapidly accept electrons from an enzyme to accelerate the kinetics of electrooxidation or electroreduction of an analyte in the presence of an enzyme or another analyte-specific redox catalyst. For example, a redox mediator may accelerate the electrooxidation of glucose in the presence of glucose oxidase or PQQ-glucose dehydrogenase, a process that can be useful for the selective assay of glucose in the presence of other electrochemically oxidizable species. Compounds having the formula 1 are examples of transition metal complexes of the present invention.



[0024] M is a transition metal and is typically iron, cobalt, ruthenium, osmium, or vanadium. Ruthenium and osmium

are particularly suitable for redox mediators.

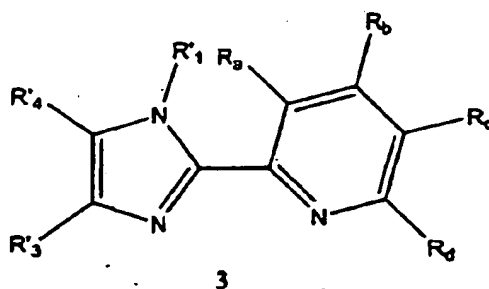
[0025] L is a bidentate ligand containing at least one imidazole ring. One example of L is a 2,2'-biimidazole having the following structure 2:



[0026] R_1 and R_2 are H or substituents attached to two of the 2,2'-biimidazole nitrogens and are independently substituted or unsubstituted alkyl, alkenyl, or aryl groups. Generally, R_1 and R_2 are unsubstituted C1 to C12 alkyls. Typically, R_1 and R_2 are unsubstituted C1 to C4 alkyls. In some embodiments, both R_1 and R_2 are methyl.

[0027] R_3 , R_4 , R_5 , and R_6 are substituents attached to carbon atoms of the 2,2'-biimidazole and are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl. Alternatively, R_3 and R_4 in combination or R_5 and R_6 in combination independently form a saturated or unsaturated 5- or 6-membered ring. An example of this is a 2,2'-bibenzoimidazole derivative. Typically, the alkyl and alkoxy portions are C1 to C12. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. Generally, R_3 , R_4 , R_5 , and R_6 are independently -H or unsubstituted alkyl groups. Typically, R_3 , R_4 , R_5 , and R_6 are -H or unsubstituted C1 to C12 alkyls. In some embodiments, R_3 , R_4 , R_5 , and R_6 are all -H.

[0028] Another example of L is a 2-(2-pyridyl)imidazole having the following structure 3:



R'_1 is H or a substituted or unsubstituted aryl alkenyl or alkyl. Generally, R'_1 is a substituted or unsubstituted C1-C12 alkyl. R'_1 is typically methyl or a C1-C12 alkyl that is optionally substituted with a reactive group.

R'_3 , R'_4 , R'_a , R'_b , R'_c , and R'_d are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl. Alternatively, R'_c and R'_d in combination or R'_3 and R'_4 in combination can form a saturated or unsaturated 5- or 6-membered ring. Typically, the alkyl and alkoxy portions are C1 to C12. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. Generally, R'_3 , R'_4 , R'_a , R'_b , R'_c and R'_d are independently -H or unsubstituted alkyl groups. Typically, R'_a and R'_c are -H and R'_3 , R'_4 , R'_b , and R'_d are -H or methyl.

c is an integer indicating the charge of the complex. Generally, c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge. For a number of osmium complexes, c is +2 or +3.

X represents counter ion(s). Examples of suitable counter ions include anions, such as halide (*eg.*, fluoride, chloride, bromide or iodide), sulfate, phosphate, hexafluorophosphate, and tetrafluoroborate, and cations (preferably, monovalent cations), such as lithium, sodium, potassium, tetralkylammonium, and ammonium. Preferably, X is a halide, such as chloride. The counter ions represented by X are not necessarily all the same.

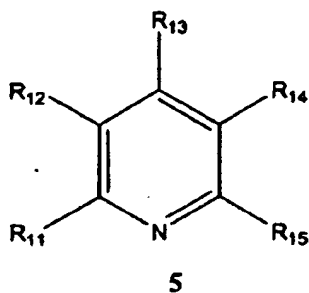
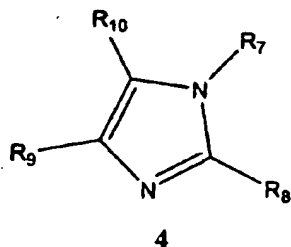
d represents the number of counter ions and is typically from 1 to 5.

L₁, L₂, L₃ and L₄ are ligands attached to the transition metal via a coordinative bond. L₁, L₂, L₃ and L₄ can be monodentate ligands or, in any combination, bi-, ter-, or tetradentate ligands. For example, L₁, L₂, L₃ and L₄ can combine to form two bidentate ligands such as, for example, two ligands selected from the group of substituted and unsubstituted 2,2'-biimidazoles, 2-(2-pyridyl)imidazoles, and 2,2'-bipyridines.

[0029] Examples of other L₁, L₂, L₃ and L₄ combinations of the transition metal complex include:

- (A) L₁ is a monodentate ligand and L₂, L₃ and L₄ in combination form a terdentate ligand;
- (B) L₁ and L₂ in combination are a bidentate ligand, and L₃ and L₄ are the same or different monodentate ligands;
- (C) L₁ and L₂ in combination, and L₃ and L₄ in combination form two independent bidentate ligands which can be the same or different; and
- (D) L₁, L₂, L₃ and L₄ in combination form a tetradentate ligand.

[0030] Examples of suitable monodentate ligands include, but are not limited to, -F, -Cl, -Br, -I, -CN, -SCN, -OH, H₂O, NH₃, alkylamine, dialkylamine, trialkylamine, alkoxy or heterocyclic compounds. The alkyl or aryl portions of any of the ligands are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. Any alkyl portions of the monodentate ligands generally contain 1 to 12 carbons. More typically, the alkyl portions contain 1 to 6 carbons. In other embodiments, the monodentate ligands are heterocyclic compounds containing at least one nitrogen, oxygen, or sulfur atom. Examples of suitable heterocyclic monodentate ligands include imidazole, pyrazole, oxazole, thiazole, pyridine, pyrazine and derivatives thereof. Suitable heterocyclic monodentate ligands include substituted and unsubstituted imidazole and substituted and unsubstituted pyridine having the following general formulas 4 and 5, respectively:



[0031] With regard to formula 4, R₇ is generally a substituted or unsubstituted alkyl, alkenyl, or aryl group. Typically, R₇ is a substituted or unsubstituted C 1 to C 12 alkyl or alkenyl. The substitution of inner coordination sphere chloride

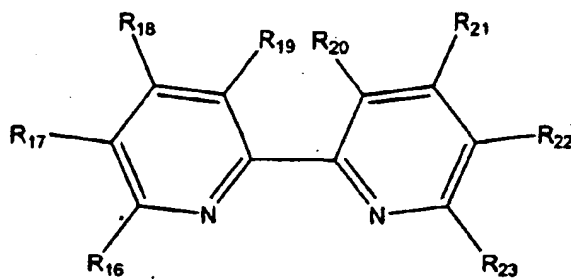
anions by imidazoles does not typically cause a large shift in the redox potential in the oxidizing direction, differing in this respect from substitution by pyridines, which typically results in a large shift in the redox potential in the oxidizing direction.

[0032] R_8 , R_9 and R_{10} are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, aryl, alkoxy-carbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, aryl-carboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl. Alternatively, R_9 and R_{10} , in combination, form a fused 5 or 6-membered ring that is saturated or unsaturated. The alkyl portions of the substituents generally contain 1 to 12 carbons and typically contain 1 to 6 carbon atoms. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. In some embodiments, R_8 , R_9 and R_{10} are -H or substituted or unsubstituted alkyl. Preferably, R_8 , R_9 and R_{10} are -H.

[0033] With regard to Formula 5, R_{11} , R_{12} , R_{13} , R_{14} and R_{15} are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, alkoxy-carbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except for aryl portions), alkoxy, alkylthio, aryl, or a reactive group. Generally, R_{11} , R_{12} , R_{13} , R_{14} and R_{15} are -H, methyl, C1-C2 alkoxy, C1-C2 alkylamino, C2-C4 dialkylamino, or a C1-C6 lower alkyl substituted with a reactive group.

[0034] One example includes R_{11} and R_{15} as -H, R_{12} and R_{14} as the same and -H or methyl, and R_{13} as -H, C1 to C12 alkoxy, -NH₂, C1 to C12 alkylamino, C2 to C24 dialkylamino, hydrazino, C1 to C12 alkylhydrazino, hydroxylamino, C1 to C12 alkoxyamino, C1 to C12 alkylthio, or C1 to C12 alkyl. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group.

[0035] Examples of suitable bidentate ligands include, but are not limited to, amino acids, oxalic acid, acetylacetone, diaminoalkanes, ortho-diaminoarenes, 2,2'-biimidazole, 2,2'-bioxazole, 2,2'-bithiazole, 2-(2-pyridyl)imidazole, and 2,2'-bipyridine and derivatives thereof. Particularly suitable bidentate ligands for redox mediators include substituted and unsubstituted 2,2'-biimidazole, 2-(2-pyridyl)imidazole and 2,2'-bipyridine. The substituted 2,2' biimidazole and 2-(2-pyridyl)imidazole ligands can have the same substitution patterns described above for the other 2,2' -biimidazole and 2-(2-pyridyl)imidazole ligand. A 2,2'-bipyridine ligand has the following general formula 6:



6

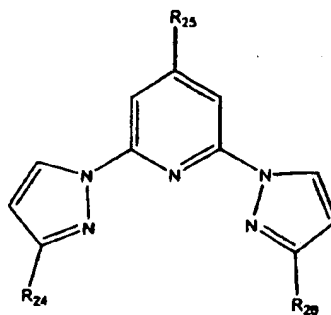
R_{16} , R_{17} , R_{18} , R_{19} , R_{20} , R_{21} , R_{22} and R_{23} are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, aryl, alkoxy-carbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, or alkyl. Typically, the alkyl and alkoxy portions are C1 to C12. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group.

[0036] Specific examples of suitable combinations of R_{16} , R_{17} , R_{18} , R_{19} , R_{20} , R_{21} , R_{22} and R_{23} include R_{16} and R_{23} as H or methyl; R_{17} and R_{22} as the same and -H or methyl; and R_{19} and R_{20} as the same and -H or methyl. An alternative combination is where one or more adjacent pairs of substituents R_{16} and R_{17} , on the one hand, and R_{22} and R_{23} , on the other hand, independently form a saturated or unsaturated 5- or 6-membered ring. Another combination includes R_{19} and R_{20} forming a saturated or unsaturated five or six membered ring.

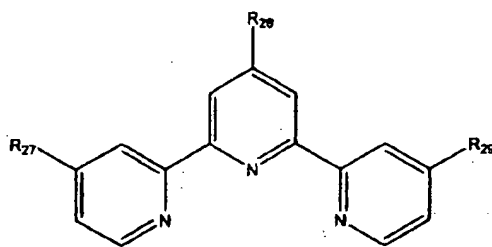
[0037] Another combination includes R_{16} , R_{17} , R_{19} , R_{20} , R_{22} and R_{23} as the same and -H and R_{18} and R_{21} as inde-

pendently -H, alkoxy, -NH₂, alkylamino, dialkylamino, alkylthio, alkenyl, or alkyl. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. As an example, R₁₈ and R₂₁ can be the same or different and are -H, C1-C6 alkyl, C1-C6 amino, C1 to C12 alkylamino, C2 to C12 dialkylamino, C1 to C12 alkylthio, or C1 to C12 alkoxy, the alkyl portions of any of the substituents are optionally substituted by a -F, -Cl, -Br, -I, aryl, C2 to C12 dialkylamino, C3 to C18 trialkylammonium, C1 to C6 alkoxy, C1 to C6 alkylthio or a reactive group.

[0038] Examples of suitable terdentate ligands include, but are not limited to, diethylenetriamine, 2,2',2"-terpyridine, 2,6-bis(N-pyrazolyl)pyridine, and derivatives of these compounds. 2,2',2"-terpyridine and 2,6-bis(N-pyrazolyl)pyridine have the following general formulas 7 and 8 respectively:



7



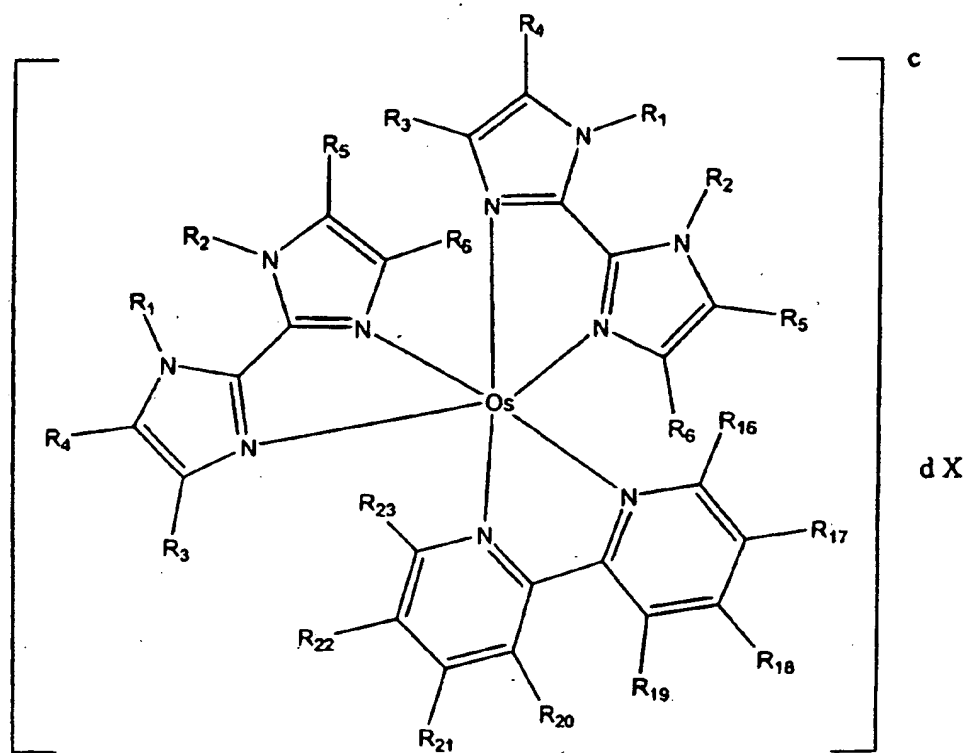
8

[0039] With regard to formula 7, R₂₄, R₂₅ and R₂₆ are independently -H or substituted or unsubstituted C1 to C12 alkyl. Typically, R₂₄, R₂₅ and R₂₆ are -H or methyl and, in some embodiments, R₂₄ and R₂₆ are the same and are -H. Other substituents at these or other positions of the compounds of formulas 7 and 8 can be added.

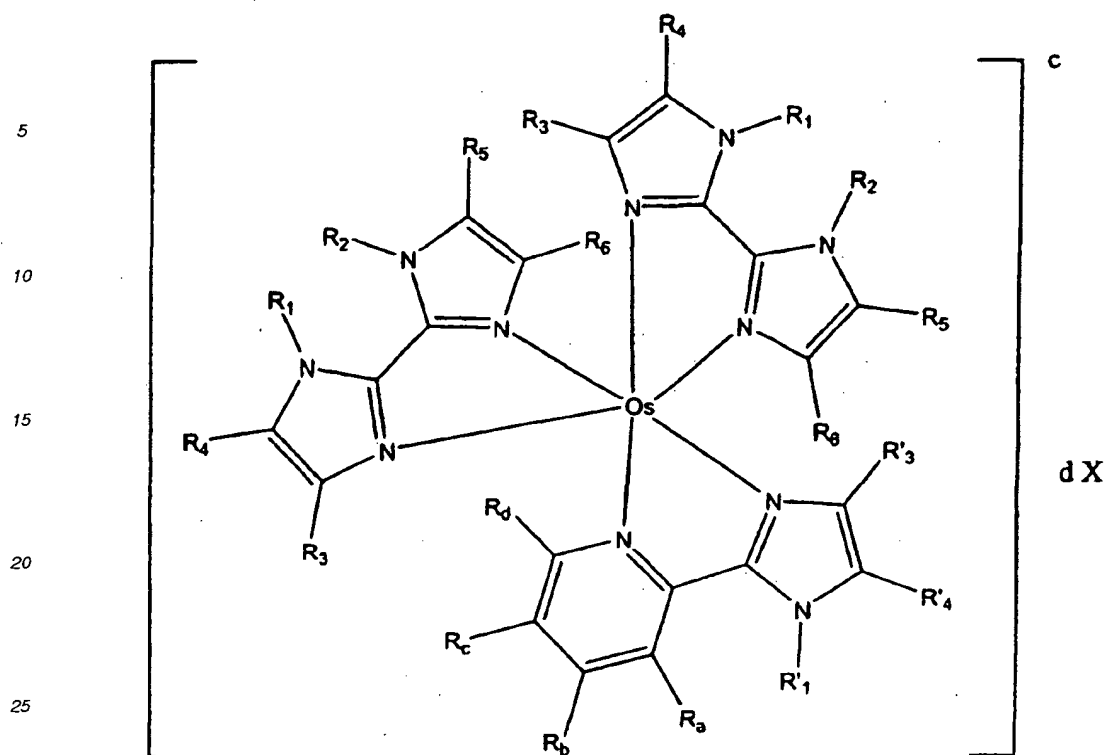
[0040] With regard to formula 8, R₂₇, R₂₈ and R₂₉ are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxylamino, alkylthio, alkenyl, aryl, or alkyl. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. Typically, the alkyl and alkoxy groups are C1 to C12 and, in some embodiments, R₂₇ and R₂₉ are the same and are -H.

[0041] Examples of suitable tetradentate ligands include triethylenetriamine, ethylenediaminediacetic acid, tetraaza macrocycles and similar compounds as well as derivatives thereof.

[0042] Examples of suitable transition metal complexes are illustrated using Formula 9 and 10:



9



10

[0043] With regard to transition metal complexes of formula 9, the metal osmium is complexed to two substituted 2,2'-biimidazole ligands and one substituted or unsubstituted 2,2'-bipyridine ligand. R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_{16} , R_{17} , R_{18} , R_{19} , R_{20} , R_{21} , R_{22} , R_{23} , c , d , and X are the same as described above.

[0044] In one embodiment, R_1 and R_2 are methyl; R_3 , R_4 , R_5 , R_6 , R_{16} , R_{17} , R_{19} , R_{20} , R_{22} and R_{23} are -H; and R_{18} and R_{21} are the same and are -H, methyl, or methoxy. Preferably, R_{18} and R_{21} are methyl or methoxy.

[0045] In another embodiment, R_1 and R_2 are methyl; R_3 , R_4 , R_5 , R_6 , R_{16} , R_{17} , R_{18} , R_{19} , R_{20} , R_{22} and R_{23} are -H; and R_{21} is halo, C1 to C12 alkoxy, C1 to C12 alkylamino, or C2 to C24 dialkylamino. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. For example, R_{21} is a C1 to C12 alkylamino or C2 to C24 dialkylamino, the alkyl portion(s) of which are substituted with a reactive group, such as a carboxylic acid, activated ester, or amine. Typically, the alkylamino group has 1 to 6 carbon atoms and the dialkylamino group has 2 to 8 carbon atoms.

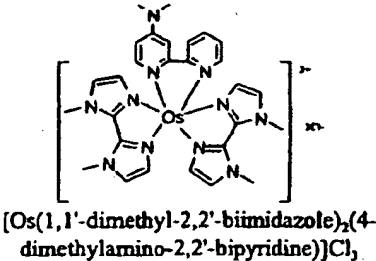
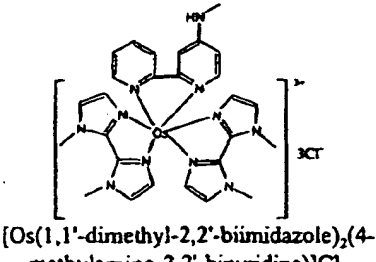
[0046] With regard to transition metal complexes of formula 10, the metal osmium is complexed to two substituted 2,2'-biimidazole ligands and one substituted or unsubstituted 2-(2-pyridyl)imidazole ligand. R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R'_1 , R'_3 , R'_4 , R_d , R_b , R_c , R_d , c , d , and X are the same as described above.

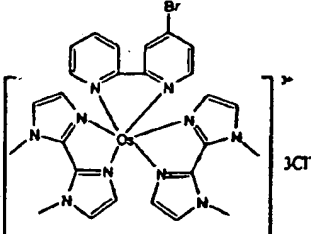
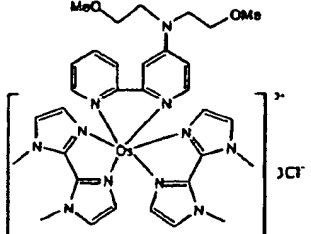
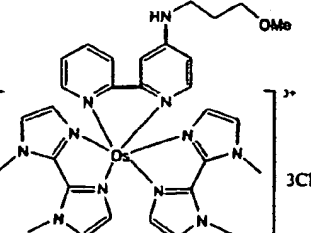
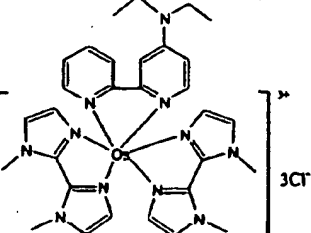
[0047] In one embodiment, R_1 and R_2 are methyl; R_3 , R_4 , R_5 , R_6 , R'_3 , R'_4 and R_d are independently -H or methyl; R_a and R_c are the same and are -H; and R_b is C1 to C12 alkoxy, C1 to C12 alkylamino, or C2 to C24 dialkylamino. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group.

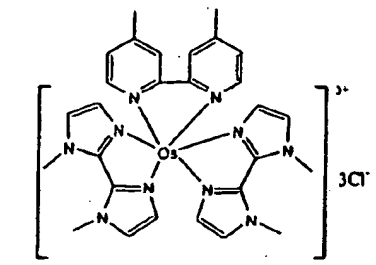
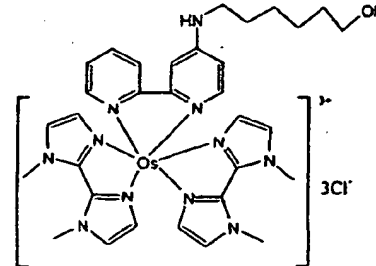
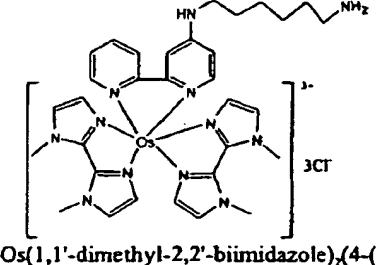
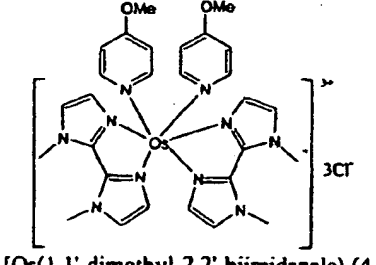
[0048] Embodiments of the invention are defined in claims 7, 8, 12-17.

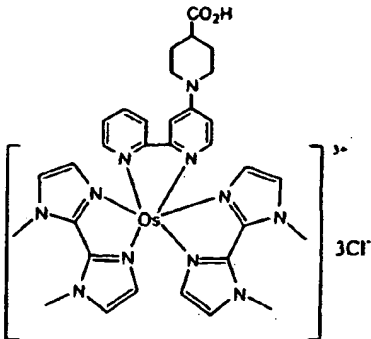
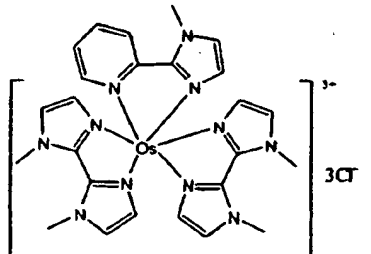
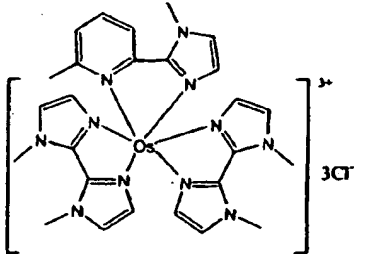
[0049] A list of specific examples of preferred transition metal complexes with respective redox potentials is shown in Table 1.

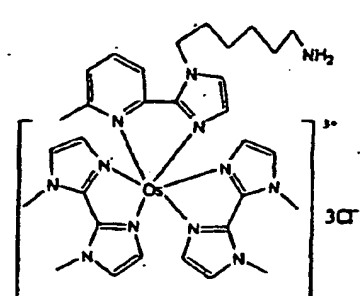
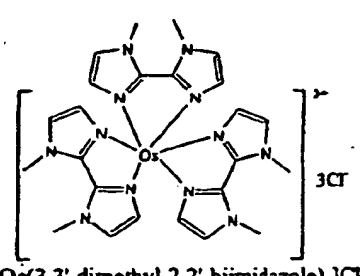
Table 1. Redox Potentials of Selected Transition Metal Complexes

Complex	Structure	$E_{1/2}$ (vs Ag/AgCl)/mV*
<u>I</u>	 <p data-bbox="542 617 915 674">[Os(1,1'-dimethyl-2,2'-biimidazole)₂(4-dimethylamino-2,2'-bipyridine)]Cl₃</p>	-110
<u>II</u>	 <p data-bbox="542 947 915 1003">[Os(1,1'-dimethyl-2,2'-biimidazole)₂(4-methylamino-2,2'-bipyridine)]Cl₃</p>	-100

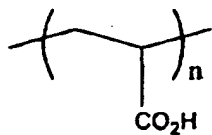
<p>5</p> <p>10</p> <p><u>III</u></p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-bromo-2,2'-bipyridine})]\text{Cl}_3$</p>	<p>128</p>
<p>20</p> <p>25</p> <p><u>IV</u></p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-di(2-methoxyethyl)amino-2,2'-bipyridine})]\text{Cl}_3$</p>	<p>-86</p>
<p>30</p> <p>35</p> <p>40</p> <p><u>V</u></p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-(3-methoxypropyl)amino-2,2'-bipyridine})]\text{Cl}_3$</p>	<p>-97</p>
<p>45</p> <p>50</p> <p>55</p> <p><u>VI</u></p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-diethylamino-2,2'-bipyridine})]\text{Cl}_3$</p>	<p>-120</p>

<p>5</p> <p>10</p> <p><u>VII</u></p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4,4'\text{-dimethyl-2,2'-bipyridine})]\text{Cl}_3$</p>	<p>32</p>
<p>20</p> <p><u>VIII</u></p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-(6-hydroxyhexyl)amino-2,2'-bipyridine})]\text{Cl}_3$</p>	<p>-100</p>
<p>30</p> <p><u>IX</u></p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-(6-aminoethyl)amino-2,2'-bipyridine})]\text{Cl}_3$</p>	<p>-93</p>
<p>45</p> <p><u>X</u></p> <p>50</p> <p>55</p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-methoxypyridine})]\text{Cl}_3$</p>	<p>-125</p>

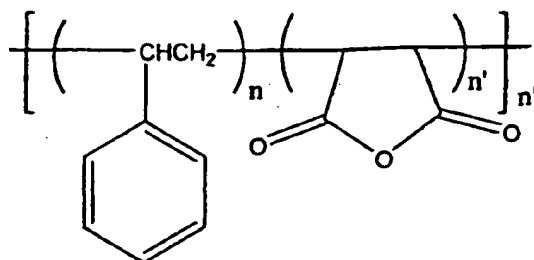
<p>XI</p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-}(\text{N-(4-carboxypiperidino)-2,2'-bipyridine}))]\text{Cl}_3$</p>	<p>-60</p>
<p>XII</p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(1\text{-methyl-2-(2-pyridyl)imidazole})]\text{Cl}_3$</p>	<p>-74</p>
<p>XIII</p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(1\text{-methyl-2-(6-methylpyrid-2-yl)imidazole})]\text{Cl}_3$</p>	<p>-97</p>

<p><u>XIV</u></p>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(1\text{-(6-aminohexyl)-2-(6-methylpyridin-2-yl)imidazole})]^{3+} 3\text{Cl}^-$</p>	<p>-81</p>
<p><u>XV</u></p>	 <p>$[\text{Os}(3,3'\text{-dimethyl-2,2'-biimidazole})_2]^{3+} 3\text{Cl}^-$</p>	<p>-230</p>
<p>* Redox potentials were estimated by averaging the positions of the reduction wave peaks and the oxidation wave peaks of cyclic voltammograms (CVs) obtained in pH 7 PBS buffer with a glassy carbon working electrode, a graphite counter electrode and a standard Ag/AgCl reference electrode at a sweep rate of 50 mV/s.</p>		

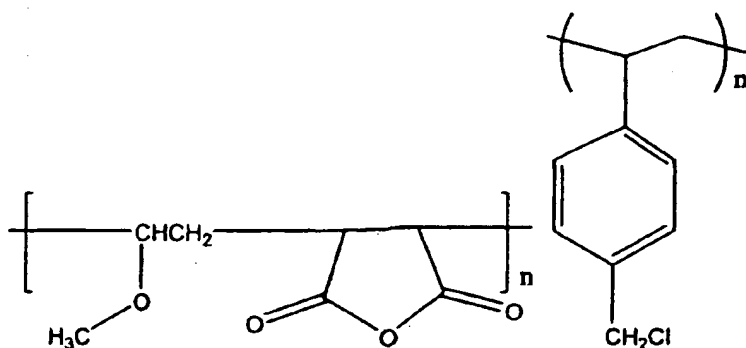
[0050] The transition metal complexes of Formula 1 also include transition metal complexes that are coupled to a polymeric backbone through one or more of L₁, L₂, L₃, and L₄. Additional examples of suitable transition metal complexes are described in WO 01/36660 and EP 1 230 248 respectively, entitled "Polymeric Transition Metal Complexes and Uses Thereof", filed on even date herewith. In some embodiments, the polymeric backbone has functional groups that act as ligands of the transition metal complex. Such polymeric backbones include, for example, poly(4-vinylpyridine) and poly(N vinylimidazole) in which the pyridine and imidazole groups, respectively, can act as monodentate ligands of the transition metal complex. In other embodiments, the transition metal complex can be the reaction product between a reactive group on a precursor polymer and a reactive group on a ligand of a precursor transition metal complex (such as complex of Formula 1 where one of L₁, L₂, L₃, and L₄ includes a reactive group, as described above). Suitable precursor polymers include, for example, poly(acrylic acid) (Formula 11), styrene/maleic anhydride copolymer (Formula 12), methylvinylether/maleic anhydride copolymer (GANTREZ polymer) (Formula 13), poly(vinylbenzylchloride) (Formula 14), poly(allylamine) (Formula 15), polylysine (Formula 16), carboxy-poly(vinylpyridine) (Formula 17), and poly(sodium 4-styrene sulfonate) (Formula 18).



11

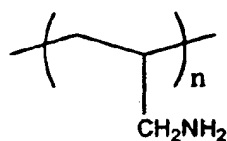


12

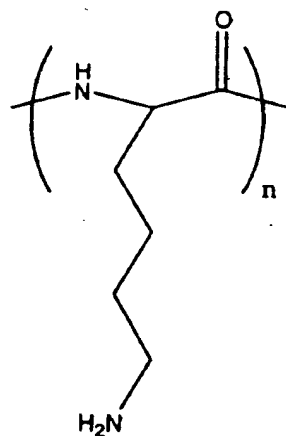


13

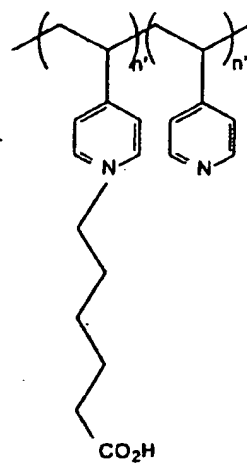
14



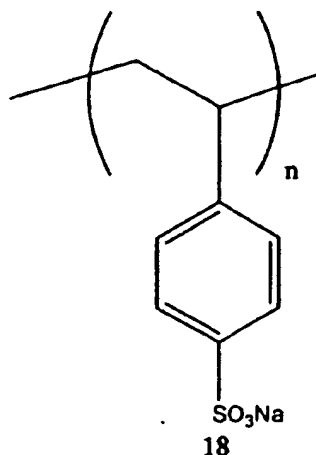
15



16



17



[0051] Alternatively, the transition metal complex can have reactive group(s) for immobilization or conjugation of the complexes to other substrates or carriers, examples of which include macromolecules (e.g., enzymes) and surfaces (e.g., electrode surfaces).

[0052] For reactive attachment to polymers, substrates, or other carriers, the transition metal complex precursor includes at least one reactive group that reacts with a reactive group on the polymer, substrate, or carrier. Typically, covalent bonds are formed between the two reactive groups to generate a linkage. Examples of such linkages are provided in Table 2, below. Generally, one of the reactive groups is an electrophile and the other reactive group is a nucleophile.

Table 2:

Examples of Reactive Group Linkages		
First Reactive Group	Second Reactive Group	Resulting Linkage
Activated ester*	Amine	Carboxamide
Acrylamide	Thiol	Thioether
Acyl azide	Amine	Carboxamide
Acyl halide	Amine	Carboxamide
Carboxylic acid	Amine	Carboxamide
Aldehyde or ketone	Hydrazine	Hydrazone
Aldehyde or ketone	Hydroxyamine	Oxime
Alkyl halide	Amine	Alkylamine
Alkyl halide	Carboxylic acid	Carboxylic ester
Alkyl halide	Imidazole	Imidazolium
Alkyl halide	Pyridine	Pyridinium
Alkyl halide	Alcohol/phenol	Ether
Alkyl halide	Thiol	Thioether
Alkyl sulfonate	Thiol	Thioether
Alkyl sulfonate	Pyridine	Pyridinium

* Activated esters, as understood in the art, generally include esters of succinimidyl, benzotriazolyl, or aryl substituted by electron-withdrawing groups such as sulfo, nitro, cyano, or halo; or carboxylic acids activated by carbodiimides.

Table 2: (continued)

Examples of Reactive Group Linkages		
First Reactive Group	Second Reactive Group	Resulting Linkage
Alkyl sulfonate	Imidazole	Imidazolium
Alkyl sulfonate	Alcohol/phenol	Ether
Anhydride	Alcohol/phenol	Ester
Anhydride	Amine	Carboxamide
Aziridine	Thiol	Thioether
Aziridine	Amine	Alkylamine
Aziridine	Pyridine	Pyridinium
Epoxide	Thiol	Thioether
Epoxide	Amine	Alkylamine
Epoxide	Pyridine	Pyridinium
Halotriazine	Amine	Aminotriazine
Halotriazine	Alcohol	Triazinyl ether
Imido ester	Amine	Amidine
Isocyanate	Amine	Urea
Isocyanate	Alcohol	Urethane
Isothiocyanate	Amine	Thiourea
Maleimide	Thiol	Thioether
Sulfonyl halide	Amine	Sulfonamide

[0053] Transition metal complexes of the present invention can be soluble in water or other aqueous solutions, or in organic solvents. In general, the transition metal complexes can be made soluble in either aqueous or organic solvents by having an appropriate counter ion or ions, X. For example, transition metal complexes with small counter anions, such as F⁻, Cl⁻, and Br⁻, tend to be water soluble. On the other hand, transition metal complexes with bulky counter anions, such as I⁻, BF₄⁻ and PF₆⁻, tend to be soluble in organic solvents. Preferably, the solubility of transition metal complexes of the present invention is greater than about 0.1 M (moles/liter) at 25°C for a desired solvent.

[0054] The transition metal complexes discussed above are useful as redox mediators in electrochemical sensors for the detection of analytes in bio-fluids. The use of transition metal complexes as redox mediators is described, for example, in U.S. Patents Nos. 5,262,035, 5,262,305, 5,320,725, 5,365,786, 5,593,852, 5,665,222, 5,972,199, and 6,143,164 and U.S. Patent Applications Serial Nos. 09/034,372, 09/070,677, 09/295,962, and 09/434,026. The transition metal complexes described herein can typically be used in place of those discussed in the references listed above. The transition metal complexes that include a polymeric backbone and are redox mediators can also be referred to as "redox polymers".

[0055] In general, the redox mediator is disposed on or in proximity to (e.g., in a solution surrounding) a working electrode. The redox mediator transfers electrons between the working electrode and an analyte. In some preferred embodiments, an enzyme is also included to facilitate the transfer. For example, the redox mediator transfers electrons between the working electrode and glucose (typically via an enzyme) in an enzyme-catalyzed reaction of glucose. Redox polymers are particularly useful for forming non-leachable coatings on the working electrode. These can be formed, for example, by crosslinking the redox polymer on the working electrode, or by crosslinking the redox polymer and the enzyme on the working electrode.

[0056] Transition metal complexes can enable accurate, reproducible and quick or continuous assays. Transition metal complex redox mediators accept electrons from, or transfer electrons to, enzymes or analytes at a high rate and also exchange electrons rapidly with an electrode. Typically, the rate of self exchange, the process in which a reduced redox mediator transfers an electron to an oxidized redox mediator, is rapid. At a defined redox mediator concentration, this provides for more rapid transport of electrons between the enzyme (or analyte) and electrode, and thereby shortens the response time of the sensor. Additionally, the novel transition metal complex redox mediators are typically stable

under ambient light and at the temperatures encountered in use, storage and transportation. Preferably, the transition metal complex redox mediators do not undergo chemical change, other than oxidation and reduction, in the period of use or under the conditions of storage, though the redox mediators can be designed to be activated by reacting, for example, with water or the analyte.

[0057] The transition metal complex can be used as a redox mediator in combination with a redox enzyme to electrooxidize or electroreduce the analyte or a compound derived of the analyte, for example by hydrolysis of the analyte. The redox potentials of the redox mediators are generally more positive (i.e. more oxidizing) than the redox potentials of the redox enzymes when the analyte is electrooxidized and more negative when the analyte is electroreduced. For example, the redox potentials of the preferred transition metal complex redox mediators used for electrooxidizing glucose with glucose oxidase or PQQ-glucose dehydrogenase as enzyme is between about - 200 mV and + 200 mV versus a Ag/AgCl reference electrode, and the most preferred mediators have redox potentials between about - 100 mV and about +100 mV versus a Ag/AgCl reference electrode

Crosslinking in Transition Metal Complex Polymers

[0058] Electron transport involves an exchange of electrons between segments of the redox polymers (e.g., one or more transition metal complexes coupled to a polymeric backbone, as described above) in a crosslinked film disposed on an electrode. The transition metal complex can be bound to the polymer backbone though covalent, coordinative or ionic bonds, where covalent and coordinative binding are preferred. Electron exchange occurs, for example, through the collision of different segments of the crosslinked redox polymer. Electrons transported through the redox polymer can originate from, for example, electrooxidation or electroreduction of an enzymatic substrate, such as, for example, the oxidation of glucose by glucose oxidase.

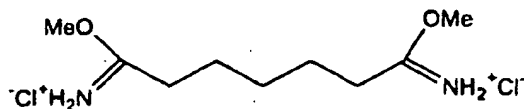
[0059] The degree of crosslinking of the redox polymer can influence the transport of electrons or ions and thereby the rates of the electrochemical reactions. Excessive crosslinking of the polymer can reduce the mobility of the segments of the redox polymer. A reduction in segment mobility can slow the diffusion of electrons or ions through the redox polymer film. A reduction in the diffusivity of electrons, for example, can require a concomitant reduction in the thickness of the film on the electrode where electrons or electron vacancies are collected or delivered. The degree of crosslinking in a redox polymer film can thus affect the transport of electrons from, for example, an enzyme to the transition metal redox centers of the redox polymer such as, for example, $\text{Os}^{2+/3+}$ metal redox centers; between redox centers of the redox polymer, and from these transition metal redox centers to the electrode.

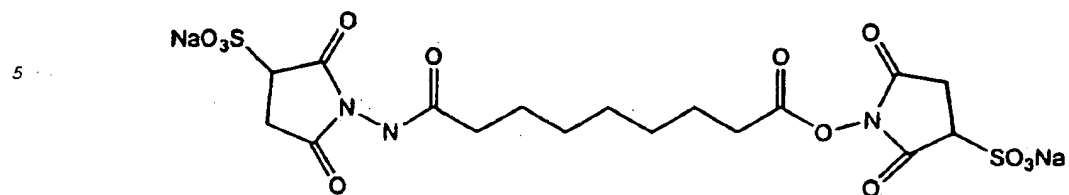
[0060] Inadequate crosslinking of a redox polymer can result in excessive swelling of the redox polymer film and to the leaching of the components of the redox polymer film. Excessive swelling can also result in the migration of the swollen polymer into the analyzed solution, in the softening of the redox polymer film, in the film's susceptibility to removal by shear, or any combination of these effects.

[0061] Crosslinking can decrease the leaching of film components and can improve the mechanical stability of the film under shear stress. For example, as disclosed in Binyamin, G. and Heller, A; *Stabilization of Wired Glucose Oxidase Anodes Rotating at 1000 rpm at 37 °C*; Journal of the Electrochemical Society, 146(8), 2965-2967, 1999 replacing a difunctional crosslinker, such as polyethylene glycol diglycidyl ether, with a trifunctional crosslinker such as *N,N*-diglycidyl-4-glycidyloxyaniline, for example, can reduce leaching and shear problems associated with inadequate crosslinking.

[0062] Examples of other bifunctional, trifunctional and tetrafunctional crosslinkers are listed below:

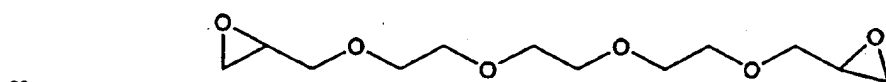
Amine-reactive Bifunctional Crosslinkers





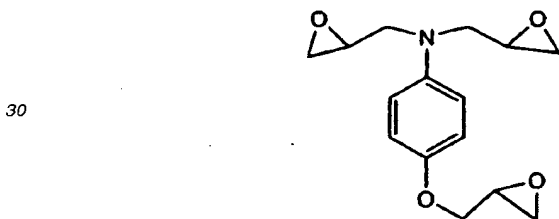
Pyridine- or Imidazole-reactive Bifunctional Crosslinkers

15



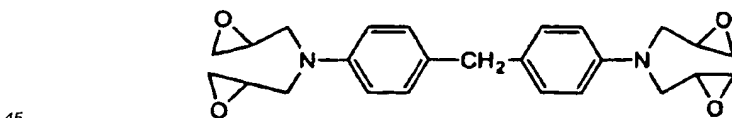
Pyridine- or Imidazole-reactive trifunctional Crosslinker

25



Pyridine- or Imidazole-reactive Tetrafunctional Crosslinkers

40



[0063] Alternatively, the number of crosslinking sites can be increased by reducing the number of transition metal complexes attached to the polymeric backbone, thus making more polymer pendant groups available for crosslinking. One important advantage of at least some of the redox polymers is the increased mobility of the pendant transition metal complexes, resulting from the flexibility of the pendant groups. As a result, in at least some embodiments, fewer transition metal complexes per polymer backbone are needed to achieve a desired level of diffusivity of electrons and current density of analyte electrooxidation or electroreduction.

Coordination in Transition Metal Complex Polymers

55

[0064] Transition metal complexes can be directly or indirectly attached to a polymeric backbone, depending on the availability and nature of the reactive groups on the complex and the polymeric backbone. For example, the pyridine groups in poly(4-vinylpyridine) or the imidazole groups in poly(N-vinylimidazole) are capable of acting as monodentate

ligands and thus can be attached to a metal center directly. Alternatively, the pyridine groups in poly(4-vinylpyridine) or the imidazole groups in poly(N-vinylimidazole) can be quaternized with a substituted alkyl moiety having a suitable reactive group, such as a carboxylate function, that can be activated to form a covalent bond with a reactive group, such as an amine, of the transition metal complex. (See Table 2 for a list of other examples of reactive groups.)

[0065] Redox centers such as, for example, $\text{Os}^{2+/3+}$ can be coordinated with five heterocyclic nitrogens and an additional ligand such as, for example, a chloride anion. An example of such a coordination complex includes two bipyridine ligands which form stable coordinative bonds, the pyridine of poly(4-vinylpyridine) which forms a weaker coordinative bond, and a chloride anion which forms the least stable coordinative bond.

[0066] Alternatively, redox centers, such as $\text{Os}^{2+/3+}$, can be coordinated with six heterocyclic nitrogen atoms in its inner coordination sphere. The six coordinating atoms are preferably paired in the ligands, for example, each ligand is composed of at least two rings. Pairing of the coordinating atoms can influence the potential of an electrode used in conjunction with redox polymers of the present invention.

[0067] Typically, for analysis of glucose, the potential at which the working electrode, coated with the redox polymer, is poised is negative of about +250 mV vs. SCE (standard calomel electrode). Preferably, the electrode is poised negative of about +150 mV vs. SCE. Poising the electrode at these potentials reduces the interfering electrooxidation of constituents of biological solutions such as, for example, urate, ascorbate and acetaminophen. The potential can be modified by altering the ligand structure of the complex.

[0068] The redox potential of a redox polymer, as described herein, is related to the potential at which the electrode is poised. Selection of a redox polymer with a desired redox potential allows tuning of the potential at which the electrode is best poised. The redox potentials of a number of the redox polymers described herein are negative of about +150 mV vs. SCE and can be negative of about +50 mV vs. SCE to allow the poising of the electrode potentials negative of about +250 mV vs. SCE and preferably negative of about +150 mV vs. SCE.

[0069] The strength of the coordination bond can influence the potential of the redox centers in the redox polymers. Typically, the stronger the coordinative bond, the more positive the redox potential. A shift in the potential of a redox center resulting from a change in the coordination sphere of the transition metal can produce a labile transition metal complex. For example, when the redox potential of an $\text{Os}^{2+/3+}$ complex is downshifted by changing the coordination sphere, the complex becomes labile. Such a labile transition metal complex may be undesirable when fashioning a metal complex polymer for use as a redox mediator and can be avoided through the use of weakly coordinating multidentate or chelating heterocyclics as ligands.

Electrode Interference

[0070] Transition metal complexes used as redox mediators in electrodes can be affected by the presence of transition metals in the analyzed sample including, for example, Fe^{3+} or Zn^{2+} . The addition of a transition metal cation to a buffer used to test an electrode results in a decline in the current produced. The degree of current decline depends on the presence of anions in the buffer which precipitate the transition metal cations. The lesser the residual concentration of transition metal cations in the sample solution, the more stable the current. Anions which aid in the precipitation of transition metal cations include, for example, phosphate. It has been found that a decline in current upon the addition of transition metal cations is most pronounced in non-phosphate buffers. If an electrode is transferred from a buffer containing a transition metal cation to a buffer substantially free of the transition metal cation, the original current is restored.

[0071] The decline in current is thought to be due to additional crosslinking of a pyridine-containing polymer backbone produced by the transition metal cations. The transition metal cations can coordinate nitrogen atoms of different chains and chain segments of the polymers. Coordinative crosslinking of nitrogen atoms of different chain segments by transition metal cations can reduce the diffusivity of electrons.

[0072] Serum and other physiological fluids contain traces of transition metal ions, which can diffuse into the films of electrodes made with the redox polymers of the present invention, lowering the diffusivity of electrons and thereby the highest current reached at high analyte concentration. In addition, transition metal ions like iron and copper can bind to proteins of enzymes and to the reaction centers or channels of enzymes, reducing their turnover rate. The resulting decrease in sensitivity can be remedied through the use of anions which complex with interfering transition metal ions, for example, in a buffer employed during the production of the transition metal complex. A non-cyclic polyphosphate such as, for example, pyrophosphate or triphosphate, can be used. For example, sodium or potassium non-cyclic polyphosphate buffers can be used to exchange phosphate anions for those anions in the transition metal complex which do not precipitate transition metal ions. The use of linear phosphates can alleviate the decrease in sensitivity by forming strong complexes with the damaging transition metal ions, assuring that their activity will be low. Other complexing agents can also be used as long as they are not electrooxidized or electroreduced at the potential at which the electrode is poised.

Enzyme Damage and its Alleviation

[0073] Glucose oxidase is a flavoprotein enzyme that catalyzes the oxidation by dioxygen of D-glucose to D-glucono-1,5-lactone and hydrogen peroxide. Reduced transition metal cations such as, for example, Fe^{2+} , and some transition metal complexes, can react with hydrogen peroxide. These reactions form destructive OH radicals and the corresponding oxidized cations. The presence of these newly formed transition metal cations can inhibit the enzyme and react with the metal complex. Also, the oxidized transition metal cation can be reduced by the FADH_2 centers of an enzyme, or by the transition metal complex.

[0074] Inhibition of the active site of an enzyme or a transition metal complex by a transition metal cation, as well as damaging reactions with OH radicals can be alleviated, thus increasing the sensitivity and functionality of the electrodes by incorporating non-cyclic polyphosphates, as discussed above. Because the polyphosphate/metal cation complex typically has a high (oxidizing) redox potential, its rate of oxidation by hydrogen peroxide is usually slow. Alternatively, an enzyme such as, for example, catalase, can be employed to degrade hydrogen peroxide.

EXAMPLES

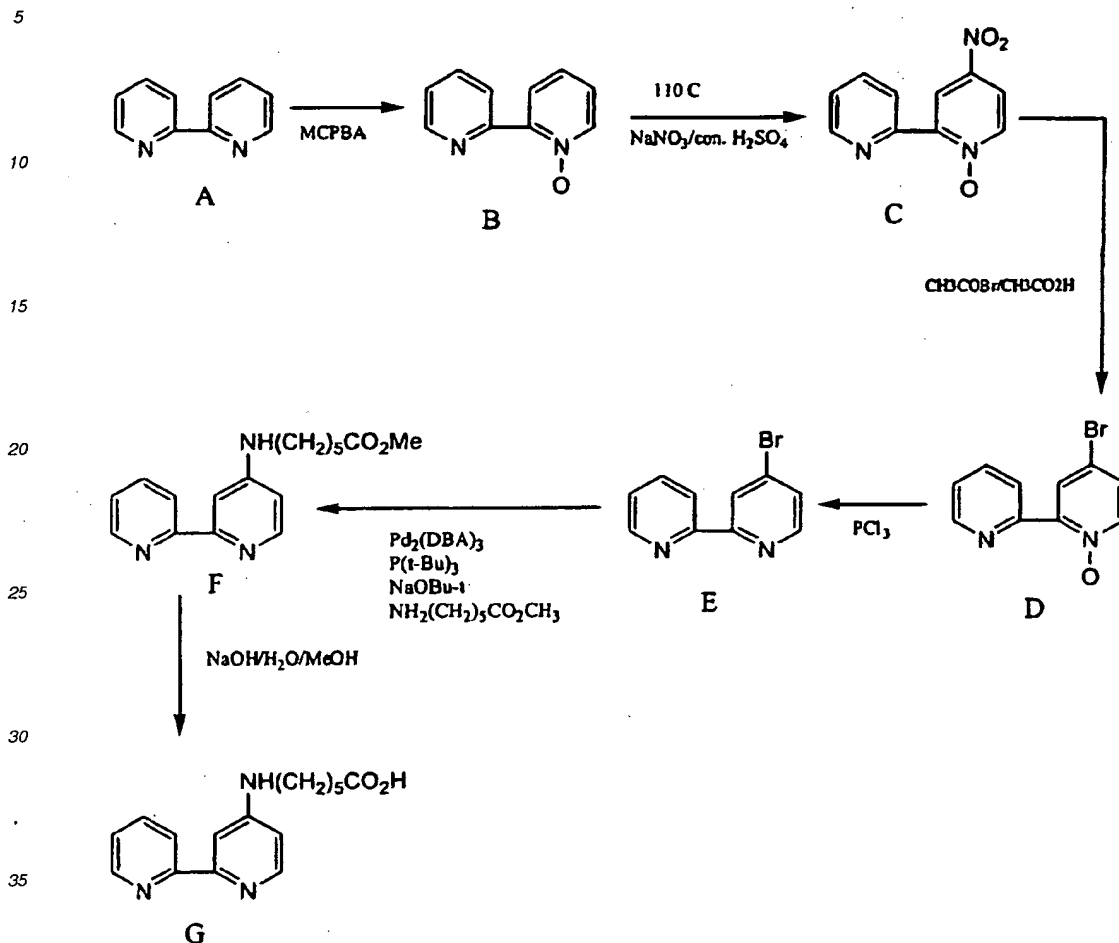
[0075] Unless indicated otherwise, all of the chemical reagents are available from Aldrich Chemical Co. (Milwaukee, WI) or other sources. Additional examples are provided in WO 01/36660 and EP 1 230 248 respectively, entitled "Polymeric Transition Metal Complexes and Uses Thereof", filed on even date herewith. For purposes of illustration, the synthesis of several transition metal complex ligands are shown below:

Example 1**Synthesis of 4-(5-carboxypentyl)amino-2,2'-bipyridyl**

[0076] This example illustrates how a carboxy reactive group is introduced onto a 2-2'-bipyridyl derivative.

Synthesis of compound D: To compound C (formed from A and B)

[0077]



according to Wenkert, D.; Woodward, R.B. *J. Org. chem.* **48**, 283(1983)) (5 g) dissolved in 30 mL acetic acid in a 100 mL round bottom flask was added 16 mL acetyl bromide. The yellow mixture was refluxed for 1.5 h and then rotovaporated to dryness. The resulting light yellow solid of D was sufficiently pure enough for the next step without further purification. Yield: 95%

[0078] **Synthesis of compound E:** To a stirred suspension of compound D in 60 mL CHCl_3 was added 12 mL PCl_3 at room temperature. The mixture was refluxed for 2 h under N_2 and then cooled to room temperature. The reaction mixture was poured into 100 mL ice/water. The aqueous layer was separated and saved. The CHCl_3 layer was extracted three times with H_2O (3x60 mL) and then discarded. The combined aqueous solution was neutralized with NaHCO_3 powder to about pH 7 to 8. The resulting white precipitate was collected by suction filtration, washed with H_2O (30 mL) and then dried under vacuum at 50°C for 24 h. Yield: 85%.

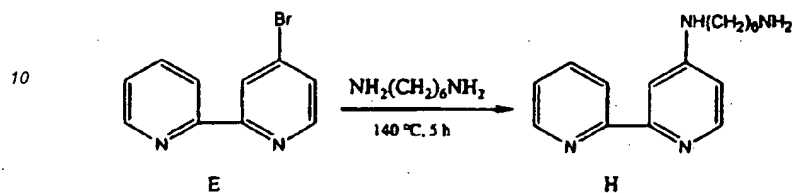
[0079] **Synthesis of compound F:** Compound F was synthesized from compound E (5 g) and 6-aminocaproic acid methyl ester (6g) using the palladium-catalyzed amination method of aryl bromides described by Hartwig et al. (Hartwig, J.F., et al. *J. Org. Chem.* **64**, 5575 (1999)). Yield: 90%.

[0080] **Synthesis of compound G:** Compound F (3 g) dissolved in 20 mL MeOH was added to a solution of NaOH (0.6g) in 30 mL H_2O . The resulting solution was stirred at room temperature for 24 h and then neutralized to pH 7 with dilute HCl. The solution was saturated with NaCl and then extracted with CHCl_3 . The CHCl_3 extract was evaporated to dryness and then purified by a silica gel column eluted with 10% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$. Yield: 70%.

Example 2

Synthesis of a 4-((6-Aminohexyl)amino)-2,2'-bipyridine:

[0081]



[0082] This example illustrates the general synthesis of a 2,2'-bipyridyl with an amine reactive group.

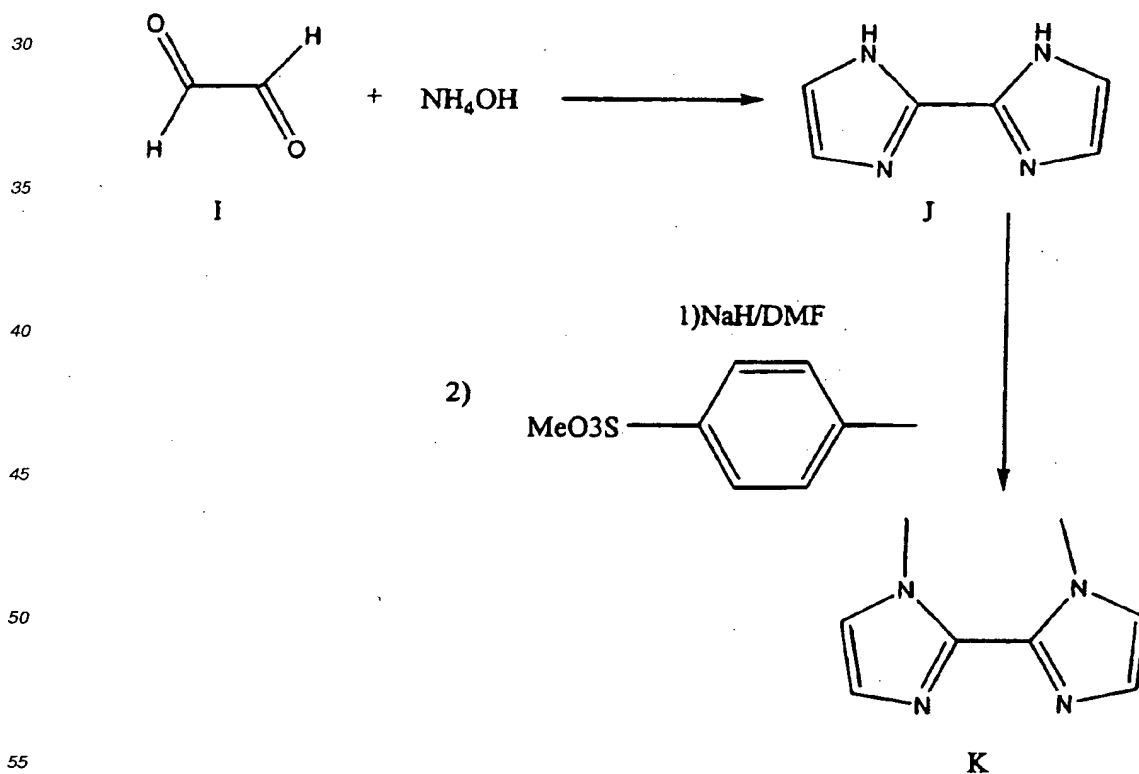
[0083] **Synthesis of compound H:** A mixture of compound E (2.5 g) and 1,6-diaminohexane (15 g) in a 250mL round bottom flask was heated under N_2 at $140^\circ C$ in an oil bath for 4-5 h. Excess 1,6-diaminohexane was removed by high vacuum distillation at $90-120^\circ C$. The product was purified by a silica gel column, eluting with 5% NH_4OH in isopropyl alcohol. Yield: 70%.

20

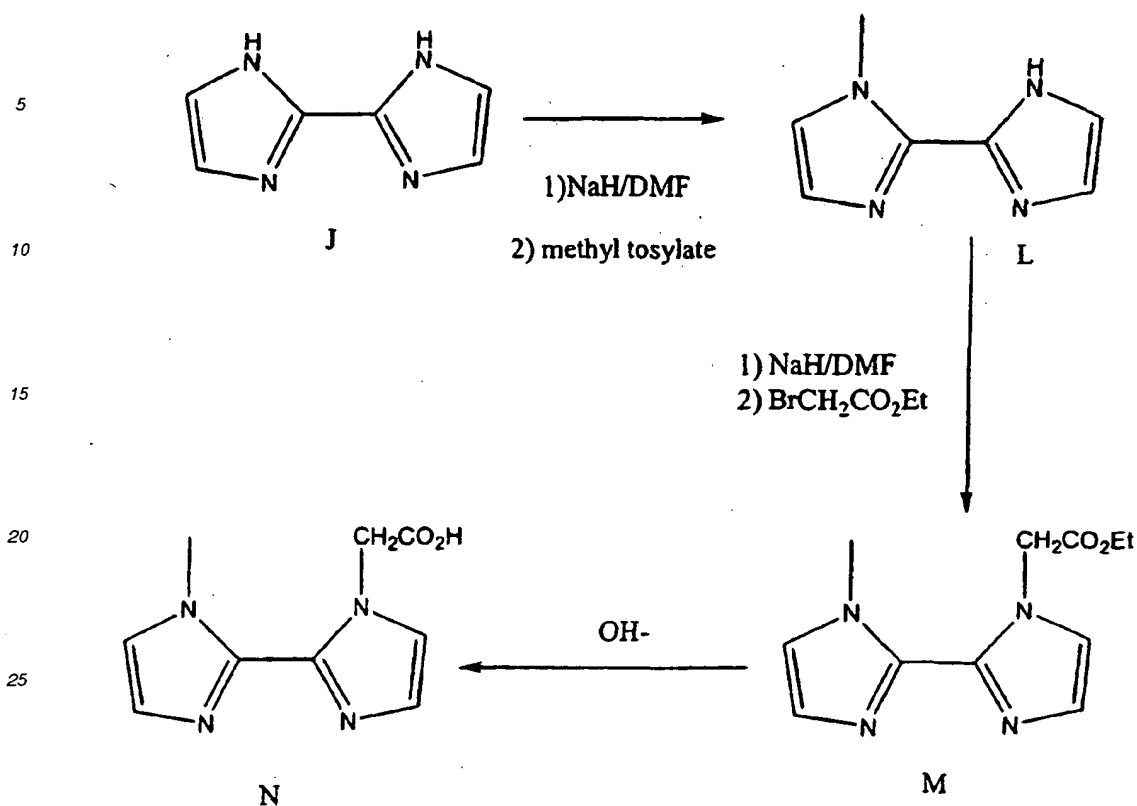
Example 3

Synthesis of 1,1'-dimethyl-2,2'-biimidazole

[0084] This example illustrates the synthesis of 2,2'-biimidazole derivatives.



[0085] The alkylation step can be carried out stepwise so two different alkyl groups can be introduced. For example:



[0086] **Synthesis of compound K:** To a stirred solution of compound J (formed from I according to Fieselmann, B. F., et al. *Inorg. Chem.* 17, 2078(1978)) (4.6g, 34.3 mmol) in 100 mL dry DMF in a 250 ml round bottom flask cooled in an ice/water bath was added in portions NaH(60% in mineral oil, 2.7 g, 68.6 mmol). After the solution was stirred at 0°C for one more hour under N₂, methyl toluenesulfonate (10.3 mL, 68.6 mmol) was added in small portions using a syringe over 30 min. The stirring of the solution in the ice/water bath was continued for 1 h and then at room temperature for 3 h. The solvent was removed by vacuum distillation. The dark residue was triturated with ether and then suction filtered and dried under vacuum. The product was purified by sublimation. Yield: 80%.

[0087] **Synthesis of compound L:** Compound L was prepared using the method described for the synthesis of compound K except that only one equivalent each of compound J, NaH and methyl toluenesulfonate was used. The product was purified by sublimation.

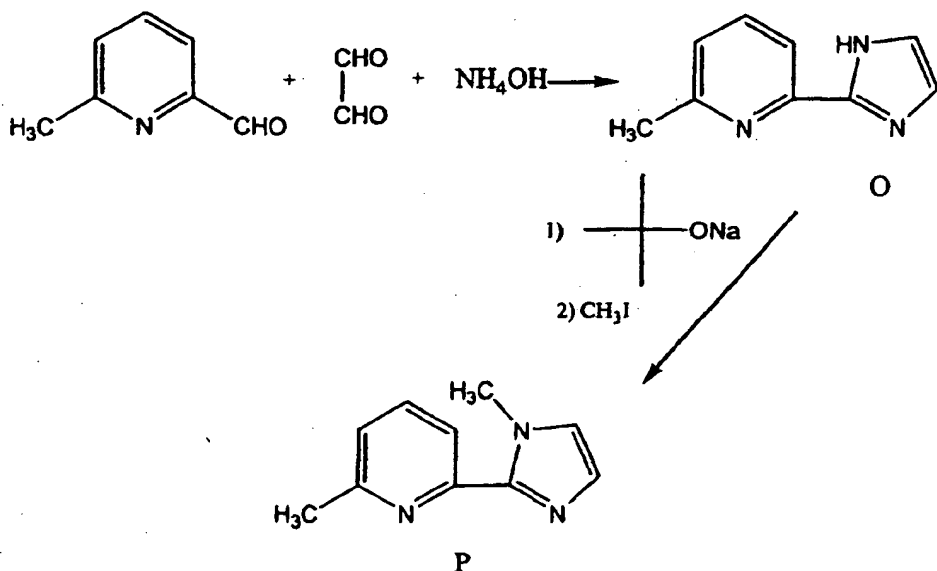
[0088] **Synthesis of compound M:** To a stirred solution of compound L (1g, 6.8 mmol) in 20 mL dry DMF in a 50 ml round bottom flask cooled in a ice/water bath is added in portions NaH(60% in mineral oil, 0.27 g, 6.8 mmol). After the solution is stirred at 0°C for one more hour under N₂, ethyl bromoacetate (0.75 mL, 6.8 mmol) is added in small portions via a syringe over 15 min. The stirring of the solution is continued in the ice/water bath for 1 h and then at room temperature for 3 h. The solvent is removed by vacuum distillation. The product is purified by a silica gel column using 10%MeOH/CHCl₃ as the eluent.

[0089] **Synthesis of Compound N:** Compound M (1g) is hydrolyzed using the method described for the synthesis of compound G. The product is purified by a silica gel column using 10% H₂O/CH₃CN as the eluent.

Example 4

Synthesis of 2-(2-Pyridyl)imidazole Heterobidentate Ligands

[0090] This example illustrates a general synthesis of heterobidentate ligands containing an imidazole ring.



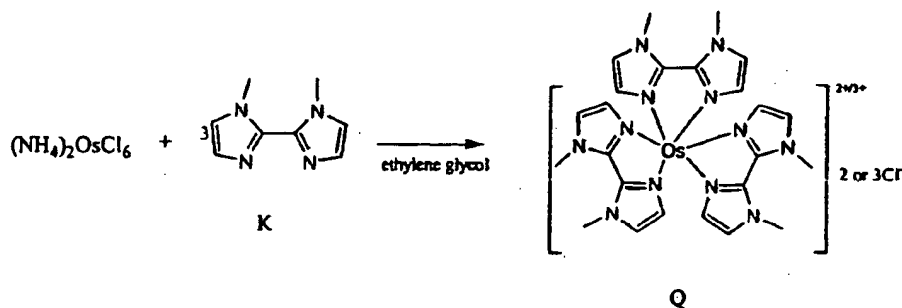
25 **[0091] Synthesis of compound O:** A solution of 6-methylpyridine-2-carboxaldehyde (26g, 0.21 mole) and glyoxal (40%, 30 mL) in 50 mL EtOH in a three-necked 250 mL round bottom flask fitted with a thermometer and an addition funnel was stirred in a NaCl/ice bath. When the solution was cooled to below 5°C, conc. NH₄OH was added dropwise through the addition funnel. The rate of the addition was controlled so that the temperature of the solution was maintained at below 5°C. After the addition, the stirring of the yellow solution was continued in the ice bath for 1 h and then at room temperature overnight. The light yellow crystals were collected by suction filtration and washed with H₂O (20 mL). The crystals were resuspended in H₂O (200 mL) and boiled briefly, followed by suction filtration, to collect the product which was dried under high vacuum. Yield: 35%.

30 **[0092] Synthesis of compound P:** Sodium t-butoxide (2g, 20.8 mmoles) was added in one portion to a stirred solution of compound O (3g, 18.9 mmoles) in 50 mL dry DMF. After all of the sodium t-butoxide was dissolved, iodomethane (1.3 mL) was added dropwise using a syringe. The stirring of the solution was continued at room temperature for 2 h and then the solution was poured into H₂O (150mL). The product was extracted with EtOAc, and the extract was dried with anhydrous Na₂SO₄ and then evaporated to give crude compound P. The product was purified by separation on a silica gel column using 10% MeOH/CHCl₃ as the eluent. Yield: 70%.

40 Example 5

Synthesis of Transition Metal Complexes with Multiple Identical Ligands

45 **[0093]** Transition metal complexes containing multiple identical bidentate or tridentate ligands can be synthesized in one step from a metal halide salt and the ligand. This example illustrates the synthesis of an osmium complex with three identical 2,2'-biimidazole bidentate ligands.

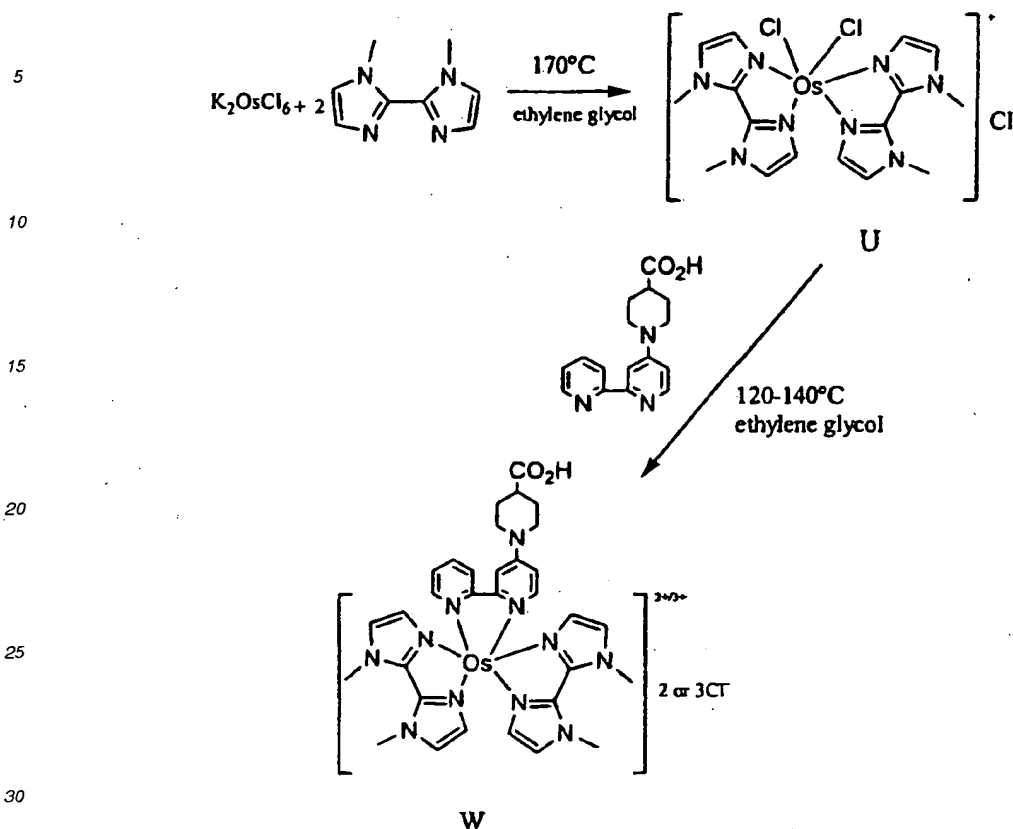


[0094] Synthesis of compound Q: Ammonium hexachloroosmate (200 mg, 0.46 mmol) and compound K (221 mg, 1.37 mmol) were mixed in 15 mL ethylene glycol in a 100 mL three-necked round bottom flask fitted with a reflux condenser. The mixture was degassed with N_2 for 15 min and then stirred under N_2 at 200-210 °C for 24 hrs. The solvent was removed by high vacuum distillation at 90-100°C. The green colored crude product was dissolved in 15 mL H_2O and stirred in air to be fully oxidized to the dark blue colored Os(III) oxidation state (about 24 h). The product was purified on a LH-20 reverse phase column using H_2O as the eluent. Yield: 50%.

Example 6

Synthesis of Transition Metal Complexes with Mixed Ligands

[0095] Transition metal complexes containing multiple types of ligands can be synthesized stepwise. First, a transition metal complex intermediate that contains one desired type of ligand and halide ligand(s), for example, chloride, is synthesized. Then the intermediate is subjected to a ligand substitution reaction to displace the halide ligand(s) with another desired type of ligand. The preparation of the following osmium complex illustrates the general synthetic scheme.

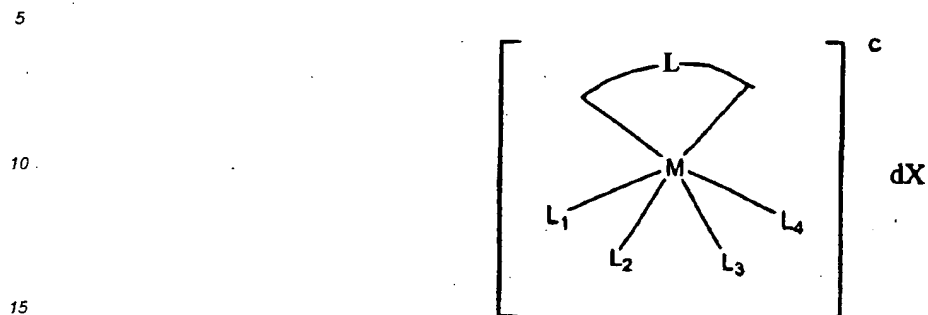


[0096] **Synthesis of Compound U:** Potassium hexachloroosmate (1g, 2.08 mmoles), compound K (0.67g, 4.16 mmoles) and LiCl (1g, 23.8 mmoles) were suspended in 40 mL ethylene glycol in a 250 mL three-necked round bottom flask fitted with a reflux condenser. The suspension was degassed with N_2 for 15 min and then stirred under N_2 at $170^\circ C$ in an oil bath for 7-8 h, resulting in a dark brown solution. The solvent was removed by high vacuum distillation at $90-100^\circ C$ bath temperature. The gummy solid was triturated with acetone twice (2x50mL) and then with H_2O once (50mL). The product was dried at $50^\circ C$ under high vacuum for 24 h.

[0097] **Synthesis of compound W:** A suspension of compound U (119mg, 0.192 mmole) and 4-(4-carboxypiperidino)amino-2,2'-bipyridyl (prepared from compound E and ethyl isonipecotote using the synthetic methods for compounds F and G) was made in 10 mL ethylene glycol in a 100mL three-necked round bottom flask equipped with a reflux condenser. The suspension was degassed with N_2 for 15 min and then stirred under N_2 at $130^\circ C$ in an oil bath for 24 h. The dark brown solution was cooled to room temperature and then poured into EtOAc (50mL). The precipitate was collected by suction filtration. The dark brown solid thus obtained was compound W with osmium in a $2+$ oxidation state. For ease of purification, the osmium $2+$ complex was oxidized to an osmium $3+$ complex by dissolving the dark brown solid in 20 mL H_2O and stirring the solution in open air for 24 h. The resulting dark green solution was poured into a stirred solution of NH_4PF_6 (1g) in 20 mL H_2O . The resulting dark green precipitate of $[Os(1,1'-dimethyl-2,2'-biimidazole)_2(4-(4-carboxypiperidino)amino-2,2'-bipyridyl)]^{3+}3PF_6^-$ was collected by suction filtration and washed with 5 mL H_2O and then dried at $40^\circ C$ under high vacuum for 48 h. The counter anion PF_6^- of $[Os(1,1'-dimethyl-2,2'-biimidazole)_2(4-(4-carboxypiperidino)amino-2,2'-bipyridyl)]^{3+}3PF_6^-$ was exchanged to the more water soluble chloride anion. A suspension of the PF_6^- salt of compound W (150mg) and Cl^- resin (10 mL) in H_2O (20 mL) was stirred for 24 h, at the end of which period all of osmium complex was dissolved. The dark green solution was separated by suction filtration and then lyophilized to give compound W.

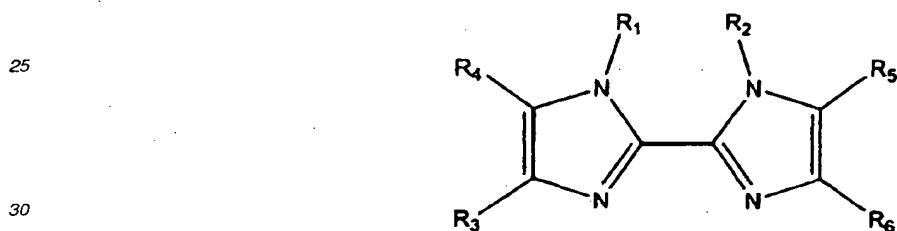
Claims

- 1. A transition metal complex having the formula:**

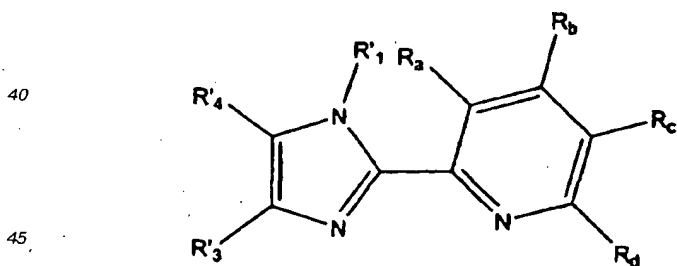


wherein

M is cobalt, iron, ruthenium, osmium, or vanadium;
L is selected from the group consisting of:

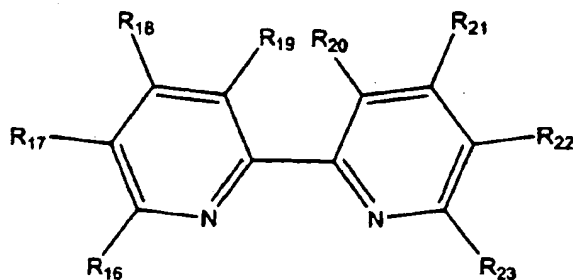


and



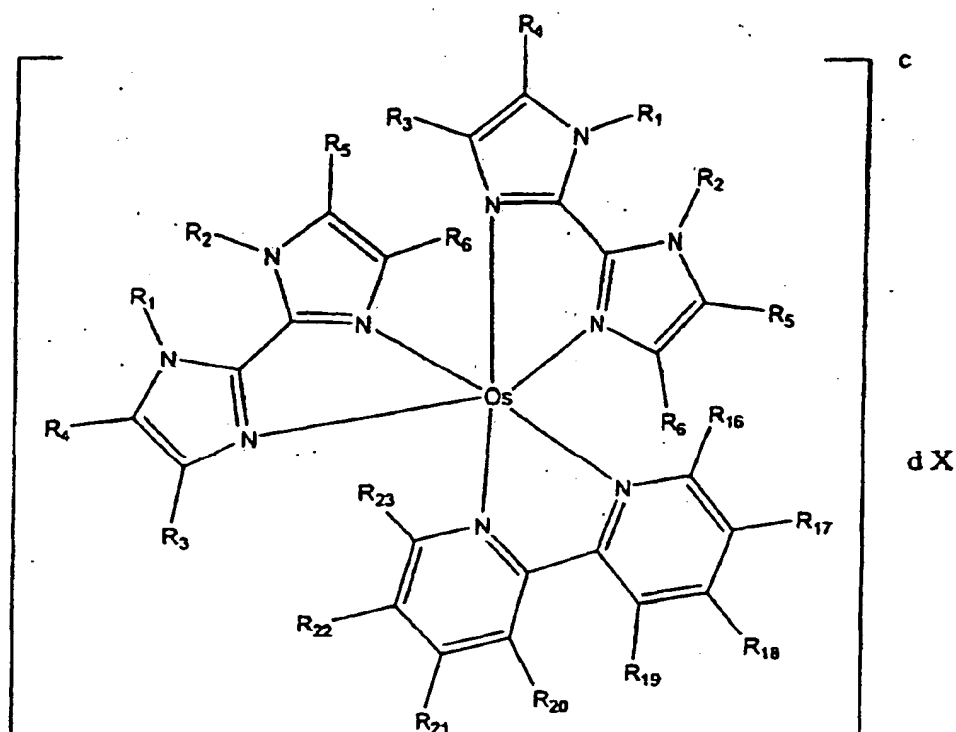
R₁, R₂, and R'₁ are independently substituted or unsubstituted alkyl, alkenyl, or aryl groups; R₃, R₄, R₅, R₆, R'₃, R'₄, R_a, R_b, R_c, and R_d are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂, or substituted or unsubstituted alkoxy carbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkoxy, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxy lamino, alkylthio, alkenyl, aryl, or alkyl; c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge; X represents at least one counter ion; d is an integer from 1 to 5 representing the number of counter ions, X; and L₁, L₂, L₃ and L₄ are ligands.

2. The transition metal complex of claim 1, wherein both R_1 , R_1' , and R_2 are unsubstituted C1 to C12 alkyl.
3. The transition metal complex of claim 1, wherein R_3 , R_4 , R_5 and R_6 are -H.
4. The transition metal complex of claim 1, wherein L_1 is a monodentate ligand.
5. The transition metal complex of claim 1, wherein L_1 comprises a heterocyclic compound containing at least one nitrogen atom.
6. The transition metal complex of claim 1, wherein one or more L_1 , L_2 , L_3 and L_4 comprises a heterocyclic compound coupled to a polymeric backbone.
7. The transition metal complex of claim 6, wherein L_1 and L_2 in combination form a bidentate ligand.
8. The transition metal complex of claim 1, wherein L_1 and L_2 in combination form a bidentate ligand.
9. The transition metal complex of claim 8, wherein the bidentate ligand comprises a 2,2'-bipyridine having the following formula:



wherein R_{16} , R_{17} , R_{18} , R_{19} , R_{20} , R_{21} , R_{22} and R_{23} are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂, or substituted or unsubstituted alkoxy carbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkoxy, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamine, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl.

10. The transition metal complex of claim 1, wherein L_1 and L_2 in combination form a first bidentate ligand and L_3 and L_4 in combination form a second bidentate ligand.
11. The transition metal complex of claim 10, wherein at least one of the first and second bidentate ligands is selected from the group consisting of substituted and unsubstituted 2,2'-bipyridines, 2,2'-biimidazoles, and 2-(2-pyridyl)imidazoles.
12. The transition metal complex of claim 1, wherein M is osmium and the transition metal complex has the following formula:



wherein

R₃, R₄, R₅, R₆, R₁₆, R₁₇, R₁₉, R₂₀, R₂₂ and R₂₃ are -H;

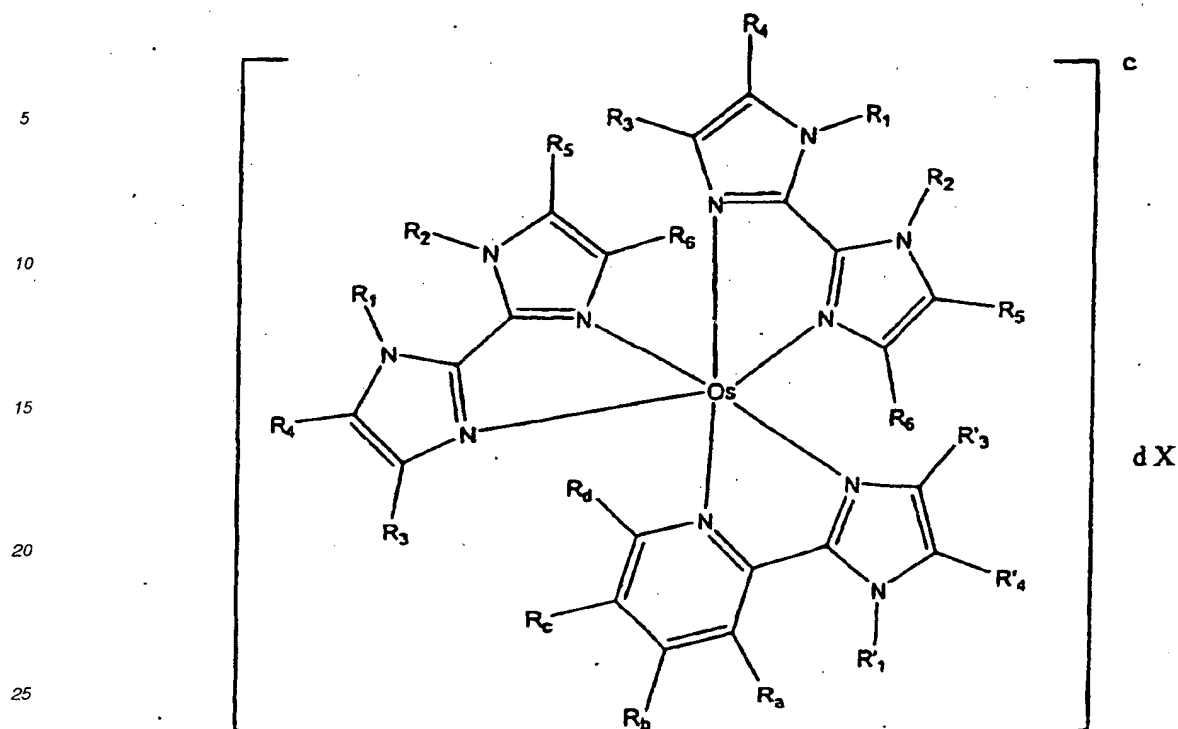
R₁ and R₂ are independently substituted or unsubstituted C1 to C12 alkyls; and

R₁₈ and R₂₁ are independently -H or substituted or unsubstituted C1-C12 alkoxy C1-C12 alkylthio, C1-C12 alkylamino, C2-C24 dialkylamino, or C1-C12 alkyl.

13. The transition metal complex of claim 12, wherein at least one of R₁, R₂, R₁₈ and R₂₁ comprises a reactive group selected from the group consisting of carboxy, activated ester including esters of succinimidyl, benzotriazolyl, or of aryl substituted by electron-withdrawing groups such as sulfo, nitro, cyano or halogen groups or carboxylic acids activated by carbodimides sulfonyl halide, sulfonate ester, isocyanate, isothiocyanate, epoxide, aziridine, halide, aldehyde, ketone, amine, acrylamide, thiol, acyl azide, acyl halide, hydrazine, hydroxylamine, alkyl halide, imidazole, pyridine, phenol, alkyl sulfonate, halotriazine, imido ester, maleimide, hydrazide, hydroxy, and photo-reactive azide aryl groups.

14. The transition metal complex of claim 12, wherein at least one of R₁, R₂, R₁₈, and R₂₁ is coupled to a polymeric backbone.

15. The transition metal complex of claim 1, wherein M is osmium and the transition metal complex has the following formula:



30 $R_3, R_4, R_5, R_6, R'_3, R'_4, R_a,$ and R_c are -H;

R_d is -H or methyl;

R_b is -H or substituted or unsubstituted C1-C12 alkoxy, C1-C12 alkylthio, C1-C12 alkylamino, C2-C24 di-alkylamino, or C1-C12 alkyl; and

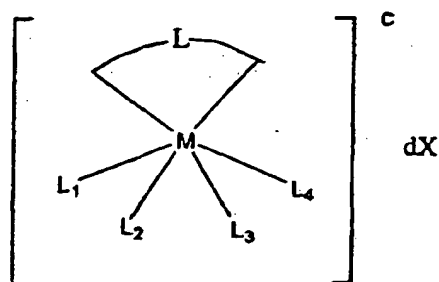
35 R_1, R'_1 and R_2 are independently substituted or unsubstituted C1 to C12 alkyl.

40 16. The transition metal complex of claim 15, wherein at least one of $R_1, R_2,$ and R'_1 comprises a reactive group selected from the group consisting of carboxy, activated ester including esters of succinimidyl, benzotriazolyl, or of aryl substituted by electron- withdrawing groups such as sulfo, nitro, cyano or halogen groups or carboxylic acids activated by carbodiimides sulfonyl halide, sulfonate ester, isocyanate, isothiocyanate, epoxide, aziridine, halide, aldehyde, ketone, amine, acrylamide, thiol, acyl azide, acyl halide, hydrazine, hydroxyamine, alkyl halide, imidazole, pyridine, phenol, alkyl sulfonate, halotriazine, imido ester, maleimide, hydrazide, hydroxy, and photo-reactive azido aryl groups.

45 17. The transition metal complex of claim 15, wherein at least one of $R_1, R_2,$ and R'_1 is coupled to a polymeric backbone.

50 18. Use of a transition metal complex having the formula

55



wherein

M is iron, cobalt, ruthenium, osmium, or vanadium;

L is a bidentate ligand comprising at least one imidazole ring;

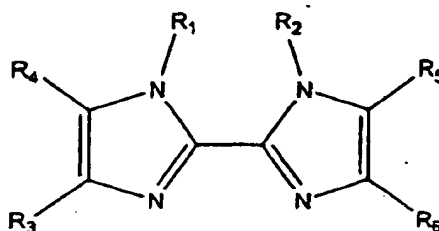
c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge;

X represents at least one counter ion;

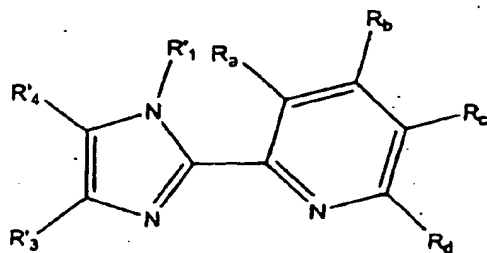
d is an integer from 1 to 5 representing the number of counter ions, X; and

L₁, L₂, L₃ and L₄ are ligands, as redox mediator.

19. The use of claim 18, wherein L is selected from the group consisting of:



and



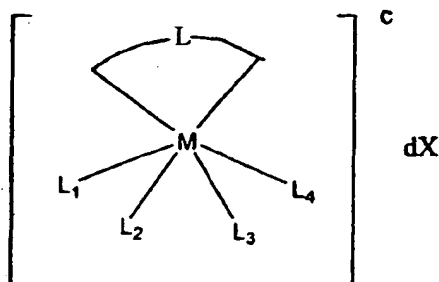
wherein

R₁, R₂, and R'₁ are independently -H or substituted or unsubstituted alkyl, alkenyl, or aryl; and

R₃, R₄, R₅, R₆, R'₃, R'₄, R_a, R_b, R_c, and R_d are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂, or substituted or unsubstituted alkoxy carbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkoxy, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl.

20. The use of claim 18, wherein L_1 comprises a heterocyclic compound containing at least one nitrogen atom.
21. The use claim 18, wherein one or more of L , L_1 , L_2 , L_3 and L_4 comprises a heterocyclic compound coupled to a polymeric backbone.
22. The use of claim 18, wherein at least one of L , L_1 , L_2 , L_3 and L_4 is coupled to a polymeric backbone.
23. A polymer comprising:

a polymeric backbone; and
a transition metal complex having the following formula:



wherein

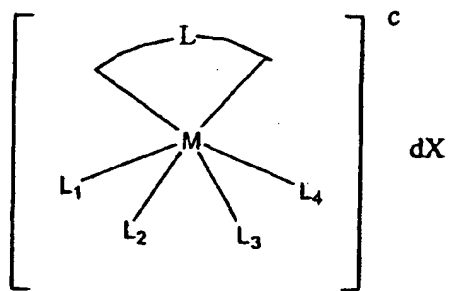
M is iron, cobalt, ruthenium, osmium, or vanadium;
 L is a bidentate ligand comprising at least one imidazole ring;
 c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge;
 X represents at least one counter ion;
 d is an integer from 1 to 5 representing the number of counter ions, X ; and
 L_1 , L_2 , L_3 and L_4 are ligands.

wherein at least one of L , L_1 , L_2 , L_3 and L_4 is coupled to the polymeric backbone.

24. The polymer of claim 23, wherein the polymer backbone is selected from the group consisting of styrene/maleic anhydride copolymer, methylvinylether/maleic anhydride copolymer, poly(4-vinylbenzylchloride) copolymer, poly(allylamine) copolymer, poly(4-vinylpyridine) copolymer, poly(4-vinylpyridine), poly(N-vinylimidazole), and poly(4-styrene sulfonate).

25. A sensor comprising:

a working electrode;
a counter electrode; and
a redox mediator disposed proximate to the working electrode, the redox mediator having the formula:



15 wherein

M is iron, cobalt, ruthenium, osmium, or vanadium;

L is a bidentate ligand comprising at least one imidazole ring;

c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge;

X represents at least one counter ion;

20 d is an integer from 1 to 5 representing the number of counter ions, X; and

L₁, L₂, L₃ and L₄ are ligands.

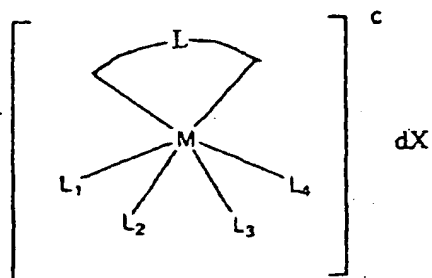
26. The sensor of claim 25, further comprising an enzyme disposed proximate to the working electrode.

27. The sensor of claim 25, wherein at least one of L, L₁, L₂, L₃ and L₄ is coupled to a polymeric backbone.

28. The sensor of claim 25, wherein the redox mediator is non-leachably disposed on the working electrode.

30 Patentansprüche

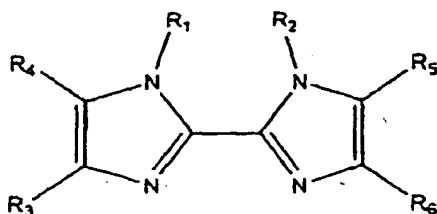
1. Übergangsmetallkomplex mit der Formel:



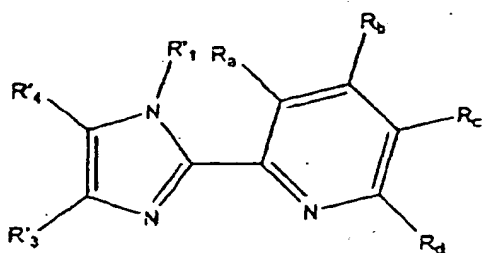
worin

50 M Cobalt, Eisen, Ruthenium, Osmium oder Vanadium ist;

L ausgewählt ist aus der Gruppe, die besteht aus:



und



R_1 , R_2 und R'_1 unabhängig voneinander substituierte oder unsubstituierte Alkyl-, Alkenyl- oder Arylgruppen sind;

R_3 , R_4 , R_5 , R_6 , R'_3 , R'_4 , R_a , R_b , R_c und R_d unabhängig voneinander -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂ oder substituiertes oder unsubstituiertes Alkoxy-carbonyl, Alkylaminocarbonyl, Dialkylaminocarbonyl, Alkoxy, Alkylamino, Dialkylamino, Alkanoylamino, Arylcarboxamido, Hydrazino, Alkylhydrazino, Hydroxylamino, Alkoxy-lamino, Alkylthio, Alkenyl, Aryl oder Alkyl sind;

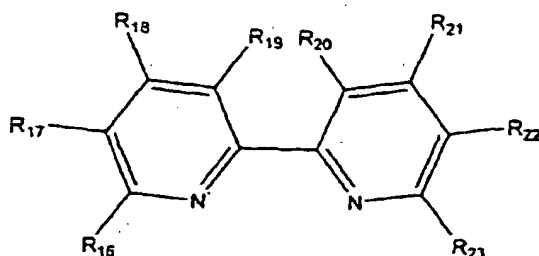
c eine ganze Zahl ist, die ausgewählt ist aus -1 bis -5 oder +1 bis +5 und eine positive oder negative Ladung bezeichnet;

X wenigstens ein Gegenion bezeichnet;

d eine ganze Zahl von 1 bis 5 ist, die die Zahl der Gegenionen X bezeichnet; und

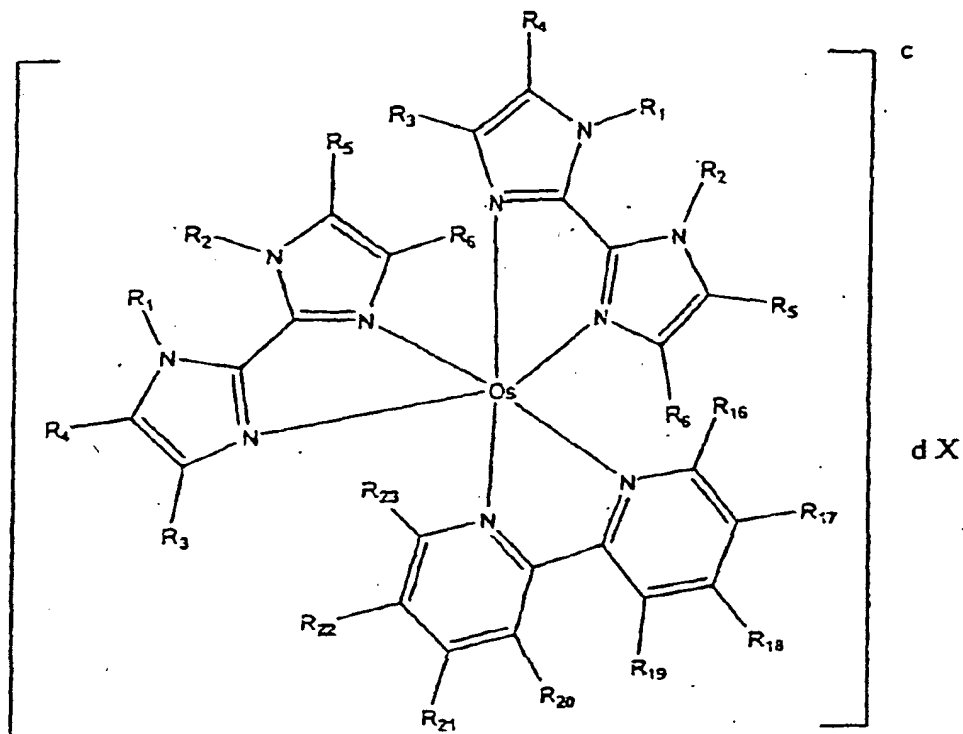
L_1 , L_2 , L_3 und L_4 Liganden sind.

2. Übergangsmetallkomplex nach Anspruch 1, wobei sowohl R_1 , R'_1 und R_2 unsubstituiertes C₁ bis C₁₂ Alkyl sind.
3. Übergangsmetallkomplex nach Anspruch 1, wobei R_3 , R_4 , R_5 und R_6 -H sind.
4. Übergangsmetallkomplex nach Anspruch 1, wobei L_1 ein einzahniger Ligand ist.
5. Übergangsmetallkomplex nach Anspruch 1, wobei L_1 eine heterocyclische Verbindung umfaßt, die wenigstens ein Stickstoffatom enthält.
6. Übergangsmetallkomplex nach Anspruch 1, wobei einer oder mehrere von L , L_1 , L_2 , L_3 und L_4 eine heterocyclische Verbindung umfassen, die an eine polymere Hauptkette gekuppelt ist.
7. Übergangsmetallkomplex nach Anspruch 6, wobei L_1 und L_2 in Kombination einen zweizahnigen Liganden bilden.
8. Übergangsmetallkomplex nach Anspruch 1, wobei L_1 und L_2 in Kombination einen zweizahnigen Liganden bilden.
9. Übergangsmetallkomplex nach Anspruch 8, wobei der zweizahnige Ligand ein 2,2'-Bipyridin der folgenden Formel umfaßt:



worin R_{16} , R_{17} , R_{18} , R_{19} , R_{20} , R_{21} , R_{22} und R_{23} unabhängig voneinander -H, -F, Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂ oder substituiertes oder unsubstituiertes Alkoxy-carbonyl, Alkylaminocarbonyl, Dialkylaminocarbonyl, Alkoxy, Alkylamino, Dialkylamino, Alkanoylamino, Arylcarboxamido, Hydrazino, Alkylhydrazino, Hydroxylamino, Alkoxyamino, Alkylthio, Alkenyl, Aryl oder Alkyl sind.

10. Übergangsmetallkomplex nach Anspruch 1, wobei L_1 und L_2 in Kombination einen ersten zweizähligen Liganden bilden und L_3 und L_4 in Kombination einen zweiten zweizähligen Liganden bilden.
11. Übergangsmetallkomplex nach Anspruch 10, wobei wenigstens einer von den ersten und zweiten zweizähligen Liganden aus der Gruppe ausgewählt ist, die besteht aus substituierten und unsubstituierten 2,2'-Bipyridinen, 2,2'-Biimidazolen und 2-(2-Pyridyl)imidazolen.
12. Übergangsmetallkomplex nach Anspruch 1, worin M Osmium ist und der Übergangsmetallkomplex die folgende Formel aufweist:



worin

$R_3, R_4, R_5, R_6, R_{16}, R_{17}, R_{19}, R_{20}, R_{22}$ und R_{23} -H sind;

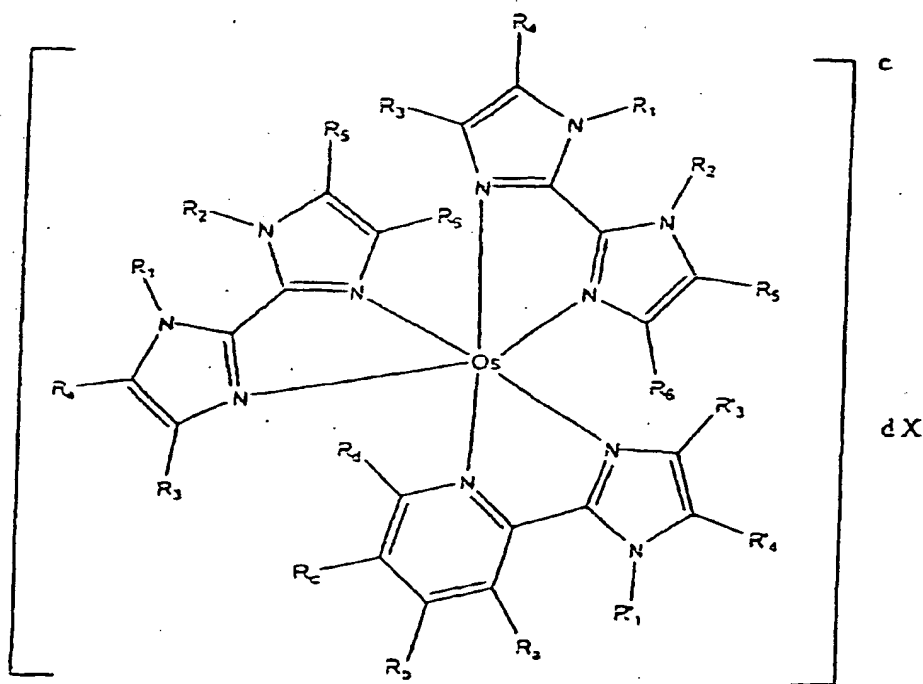
R_1 und R_2 unabhängig voneinander substituierte oder unsubstituierte C_1 bis C_{12} -Alkyle sind; und

R_{18} und R_{21} unabhängig voneinander -H oder substituiertes oder unsubstituiertes C_1 bis C_{12} Alkoxy, C_1 bis C_{12} Alkylthio, C_1 bis C_{12} -Alkylamino, C_2 bis C_{24} -Dialkylamino oder C_1 bis C_{12} Alkyl sind.

13. Übergangsmetallkomplex nach Anspruch 12, wobei wenigstens einer von R_1, R_2, R_{18} und R_{21} eine reaktive Gruppe aufweist, die aus der Gruppe ausgewählt ist, die besteht aus Carboxy, Aktivester, einschließlich Estern von Succinimidyl, Benzociazolyl oder mit elektronenanziehenden Gruppen wie Sulfo-, Nitro-, Cyano- oder Halogen-Gruppen substituiertem Aryl oder Carbonsäuren, die mit Carbodiimiden aktiviert sind, Sulfonylhalogenid, Sulfonatester, Isocyanat, Isothiocyanat, Epoxid, Aziridin, Halogenid, Aldehyd, Keton, Amin, Acrylamid, Thiol, Acylazid, Acylhalogenid, Hydrazin, Hydroxylamin, Alkylhalogenid, Imidazol, Pyridin, Phenol, Alkylsulfonat, Halogentriazin, Imidoesner, Maleimid, Hydrazid, Hydroxy und photoreaktiven Azidarylgruppen.

14. Übergangsmetallkomplex nach Anspruch 12, wobei wenigstens einer von R_1, R_2, R_{18} und R_{21} an eine polymere Hauptkette gekuppelt ist.

15. Übergangsmetallkomplex nach Anspruch 1, wobei M Osmium ist und der Übergangsmetallkomplex die folgende Formel aufweist:



worin

$R_3, R_4, R_5, R_6, R'_3, R'_4, R_a$, und R_c -H sind;

R_d -H oder Methyl ist;

R_b -H oder substituiertes oder unsubstituiertes C_1 bis C_{12} -Alkoxy, C_1 bis C_{12} Alkylthio, C_1 bis C_{12} -Alkylamino, C_2 bis C_{24} -Dialkylamino oder C_1 bis C_{12} Alkyl ist; und

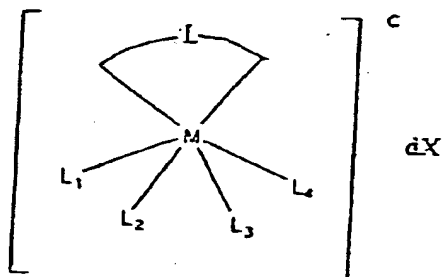
R_1, R'_1 und R_2 unabhängig voneinander substituiertes oder unsubstituiertes C_1 bis C_{12} Alkyl sind.

16. Übergangsmetallkomplex nach Anspruch 15, wobei wenigstens einer von R_1, R_2 und R'_1 eine reaktive Gruppe aufweist, die aus der Gruppe ausgewählt ist, die besteht aus Carboxy, Aktivester, einschließlich Estern von Succinimidyl, Benzotriazolyl oder mit elektronenanziehenden Gruppen wie Sulfo-, Nitro-, Cyano- oder Halogen-Gruppen

pen substituiertem Aryl oder Carbonsäuren, die mit Carbodiimiden aktiviert sind, Sulfonylhalogenid, Sulfonatester, Isocyanat, Isothiocyanat, Epoxid, Aziridin, Halogenid, Aldehyd, Keton, Amin, Acrylamid, Thiol, Acylazid, Acylhalogenid, Hydrazin, Hydroxylamin, Alkylhalogenid, Imidazol, Pyridin, Phenol, Alkylsulfonat, Halogentriazin, Imidoe-
ster, Maleimid, Hydrazid, Hydroxy und photoreaktiven Azidarylgruppen.

17. Übergangsmetallkomplex nach Anspruch 15, wobei wenigstens einer von R_1 , R_2 und R'_1 an eine polymere Hauptkette gekuppelt ist.

18. Verwendung eines Übergangsmetallkomplexes mit der Formel:



worin

M Eisen, Cobalt, Ruthenium, Osmium oder Vanadium ist;

L ein zweizähniger Ligand ist, der wenigstens einen Imidazolring aufweist;

c eine ganze Zahl ist, die aus -1 bis -5 oder +1 bis +5 ausgewählt ist und eine positive oder negative Ladung bezeichnet;

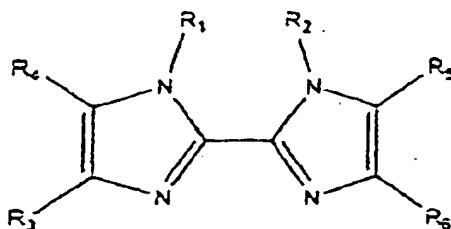
X wenigstens ein Gegenion bezeichnet;

d eine ganze Zahl von 1 bis 5 ist, die die Zahl der Gegenionen X bezeichnen; und

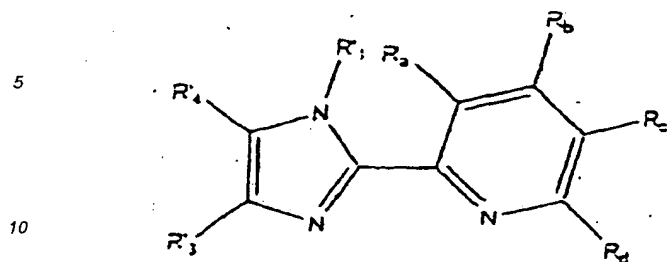
L_1 , L_2 , L_3 und L_4 Liganden sind,

als Redoxmediator.

19. Verwendung nach Anspruch 18, wobei L aus der Gruppe ausgewählt ist, die besteht aus:



und



15 worin

R_1, R_2 und R'_1 unabhängig voneinander -H oder substituiertes oder unsubstituiertes Alkyl, Alkenyl oder Aryl sind; und

$R_3, R_4, R_5, R_6, R'_3, R'_4, R_a, R_b, R_c$ und R_d unabhängig voneinander -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂ oder substituiertes oder unsubstituiertes Alkoxy-carbonyl, Alkylaminocarbo-
 20 nyl, Dialkylaminocarboxyl, Alkoxy, Alkylamino, Dialkylamino, Alkanoylamino, Arylcarboxamido, Hydrazino, Alkylhydrazino, Hydroxylamino, Alkoxyamino, Alkylthio, Alkenyl, Aryl oder Alkyl sind.

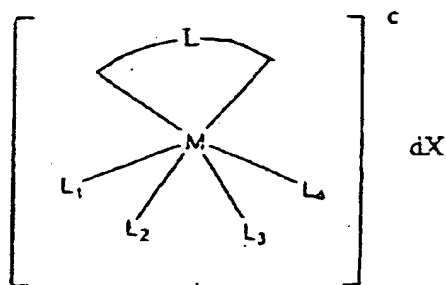
20. Verwendung nach Anspruch 18, wobei L_1 eine heterocyclische Verbindung umfaßt, die wenigstens ein Stickstoffatom enthält.

21. Verwendung nach Anspruch 18, wobei einer oder mehrere von L, L_1, L_2, L_3 und L_4 eine heterocyclische Verbindung umfaßt, die an eine polymere Hauptkette gekuppelt ist.

22. Verwendung nach Anspruch 18, wobei wenigstens einer von L, L_1, L_2, L_3 und L_4 an eine polymere Hauptkette gekuppelt ist.

23. Polymer, das umfaßt:

eine polymere Hauptkette; und
 einen Übergangsmetallkomplex mit der folgenden Formel:



55 worin

M Eisen, Cobalt, Ruthenium, Osmium oder Vanadium ist;

L ein zweizähniger Ligand ist, der wenigstens einen Imidazolring aufweist;

c eine ganze Zahl ist, die aus -1 bis -5 oder +1 bis +5 ausgewählt ist und eine positive oder negative Ladung

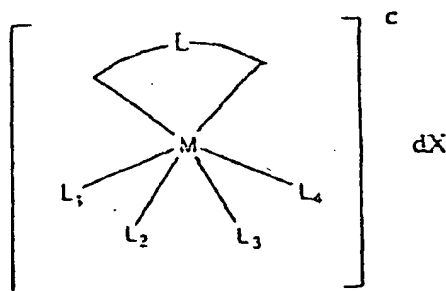
bezeichnet;
 X wenigstens ein Gegenion bezeichnet;
 d eine ganze Zahl von 1 bis 5 ist, die die Zahl der Gegenionen X bezeichnet; und
 L₁, L₂, L₃ und L₄ Liganden sind,

wobei wenigstens einer von L, L₁, L₂, L₃ und L₄ an die polymere Hauptkette gekuppelt ist.

24. Polymer nach Anspruch 23, wobei die polymere Hauptkette aus der Gruppe ausgewählt ist, die besteht aus Styrol/-Maleinsäureanhydrid-Copolymer, Methylvinylether/Maleinsäureanhydrid-Copolymer, Poly(4-vinylbenzylchlorid)-Copolymer, Poly(allylamin)-Copolymer, Poly(4-vinylpyridin)-Copolymer, Poly(4-vinylpyridin), Poly(N-vinylimidazol) und Poly(4-styrolsulfonat).

25. Sensor, der aufweist:

eine Arbeitselektrode,
 eine Gegenelektrode; und
 einen in der Nähe der Arbeitselektrode angeordneten Redoxmediator, wobei der Redoxmediator die Formel aufweist:



worin

M Eisen, Cobalt, Ruthenium, Osmium oder Vanadium ist;
 L ein zweizähniger Ligand ist, der wenigstens einen Imidazolring aufweist;
 c eine ganze Zahl ist, die aus -1 bis -5 oder +1 bis +5 ausgewählt ist und eine positive oder negative Ladung bezeichnet;
 X wenigstens ein Gegenion bezeichnet;
 d eine ganze Zahl von 1 bis 5 ist, die die Zahl der Gegenionen X bezeichnet; und
 L₁, L₂, L₃ und L₄ Liganden sind.

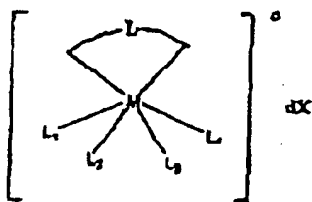
26. Sensor nach Anspruch 25, der außerdem ein Enzym aufweist, das in der Nähe der Arbeitselektrode angeordnet ist.

27. Sensor nach Anspruch 25, wobei wenigstens einer von L, L₁, L₂, L₃ und L₄ an eine polymere Hauptkette gekuppelt ist.

28. Sensor nach Anspruch 25, wobei der Redoxmediator nicht auslaugbar auf der Arbeitselektrode angeordnet ist.

Revendications

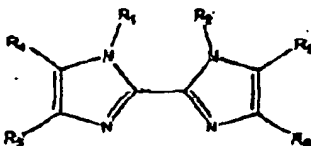
1. Complexe de métal de transition de formule :



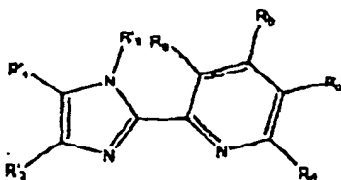
dans laquelle

M est le cobalt, le fer, le ruthénium, l'osmium, ou le vanadium ;

L est choisi dans le groupe composé de :



et



R_1 , R_2 , et R'_1 sont, indépendamment, des groupes alkyle, alcényle, ou aryle substitués ou non ;

R_3 , R_4 , R_5 , R_6 , R'_3 , R'_4 , R_a , R_b , R_c et R_d sont, indépendamment, -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂, ou des groupes alcoxy-carbonyle, alkylaminocarbonyle, dialkylaminocarbonyle, alcoxy, alkylamino, dialkylamino, alcanoylamino, aryl-carboxamido, hydrazino, alkylhydrazino, hydroxylamino, alcoxyamino, alkylthio, alcényle, aryle, ou alkyle substitués ou non ;

c est un nombre entier de -1 à -5 ou de +1 à +5 indiquant une charge positive ou négative ;

X représente au moins un contre-ion ;

d est un nombre entier de 1 à 5 représentant le nombre de contre-ions, X ; et

L_1 , L_2 , L_3 et L_4 sont des ligands.

2. Complexe de métal de transition selon la revendication 1, dans lequel à la fois R_1 , R'_1 , et R_2 sont des groupes alkyle C₁ à C₁₂ non substitués.

3. Complexe de métal de transition selon la revendication 1, dans lequel R_3 , R_4 , R_5 et R_6 sont -H.

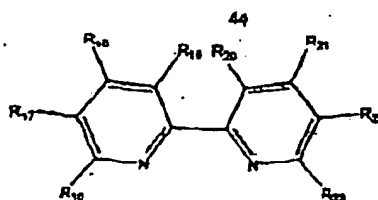
4. Complexe de métal de transition selon la revendication 1, dans lequel L_1 est un ligand monodentate.

5. Complexe de métal de transition selon la revendication 1, dans lequel L_1 comprend un composé hétérocyclique contenant au moins un atome d'azote.

6. Complexe de métal de transition selon la revendication 1, dans lequel un ou plusieurs des L, L_1 , L_2 , L_3 et L_4

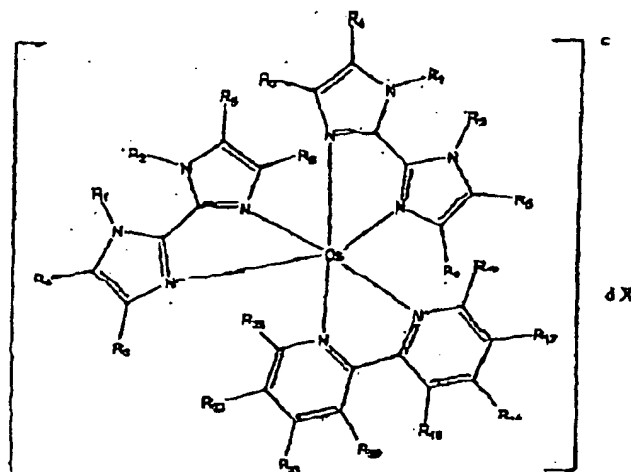
comprend (ou comprennent) un composé hétérocyclique couplé à un squelette polymère.

7. Complexe de métal de transition selon la revendication 6, dans lequel L_1 et L_2 en combinaison forment un ligand bidentate.
8. Complexe de métal de transition selon la revendication 1, dans lequel L_1 et L_2 en combinaison forment un ligand bidentate.
9. Complexe de métal de transition selon la revendication 8, dans lequel le ligand bidentate comprend une 2,2'-bipyridine répondant à la formule suivante :



dans laquelle R_{16} , R_{17} , R_{18} , R_{19} , R_{20} , R_{21} , R_{22} et R_{23} sont, indépendamment, -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂, ou des groupes alcoxy-carbonyle, alkylaminocarbonyle, dialkylaminocarbonyle, alcoxy, alkylamino, dialkylamino, alcanoylamino, aryl-carboxamido, hydrazino, alkylhydrazino, hydroxylamino, alcoxyamino, alkylthio, alcényle, aryle, ou alkyle substitués ou non.

10. Complexe de métal de transition selon la revendication 1, dans lequel L_1 et L_2 en combinaison forment un premier ligand bidentate et L_3 et L_4 en combinaison forment un second ligand bidentate.
11. Complexe de métal de transition selon la revendication 10, dans lequel au moins un des premier et second ligands bidentates est choisi dans le groupe composé des 2,2'-bipyridines, 2,2'-biimidazoles et 2-(2-pyridyl)imidazoles substitués ou non.
12. Complexe de métal de transition selon la revendication 1, dans lequel M est l'osmium et le complexe de transition répond à la formule suivante :



dans laquelle

$R_3, R_4, R_5, R_6, R_{16}, R_{17}, R_{19}, R_{20}, R_{22}$ et R_{23} sont -H ;

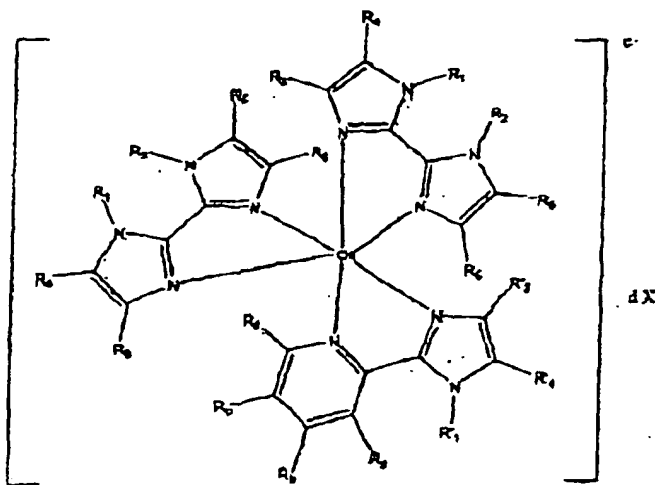
R_1 et R_2 sont, indépendamment, des groupes alkyle C_1 à C_{12} substitués ou non ; et

R_{18} et R_{21} sont, indépendamment, -H ou des groupes alcoxy C_1 à C_{12} , alkylthio C_1 à C_{12} , alkylamino C_1 à C_{12} , dialkylamino C_2 à C_{24} , ou alkyle C_1 à C_{12} , substitués ou non.

13. Complexe de métal de transition selon la revendication 12, dans lequel au moins un des R_1, R_2, R_{18} et R_{21} comprend un groupe réactif choisi dans le groupe composé des groupes carboxy, ester activé incluant les esters de succinimidyle, de benzotriazolyle ou d'aryle substitués par des groupes attracteurs d'électrons tels que des groupes sulfo, nitro, cyano ou halogènes ou des acides carboxyliques activés par des carbodiimides, halogénure de sulfonyle, ester de sulfonate, isocyanate, isothiocyanate, époxyde, aziridine, halogénure, aldéhyde, cétone, amine, acrylamide, thiol, azoture d'acyle, halogénure d'acyle, hydrazine, hydroxylamine, halogénure d'alkyle, imidazole, pyridine, phénol, sulfonate d'alkyle, halogénotriazine, imidoester, maléimide, hydrazide, hydroxy, et azidoaryle photoréactifs.

14. Complexe de métal de transition selon la revendication 12, dans lequel au moins un des R_1, R_2, R_{18} et R_{21} est couplé à un squelette polymère.

15. Complexe de métal de transition selon la revendication 1, dans lequel M est l'osmium et le complexe de transition répond à la formule suivante :



dans laquelle

$R_3, R_4, R_5, R_6, R'_3, R'_4, R_a$ et R_c sont -H ;

R_d est -H ou un groupe méthyle ;

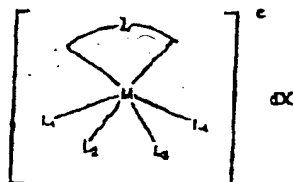
R_b est -H ou un groupe alcoxy C_1 à C_{12} , alkylthio C_1 à C_{12} , alkylamino C_1 à C_{12} , dialkylamino C_2 à C_{24} , ou alkyle C_1 à C_{12} , substitué ou non ; et

R_1, R'_1 et R_2 sont, indépendamment, un groupe alkyle C_1 à C_{12} substitué ou non.

16. Complexe de métal de transition selon la revendication 15, dans lequel au moins un des R_1, R_2 , et R'_1 comprend un groupe réactif choisi dans le groupe composé des groupes carboxy, ester activé incluant les esters de succinimidyle, de benzotriazolyle ou d'aryle substitués par des groupes attracteurs d'électrons tels que des groupes sulfo, nitro, cyano ou halogènes ou des acides carboxyliques activés par des carbodiimides, halogénure de sulfonyle, ester de sulfonate, isocyanate, isothiocyanate, époxyde, aziridine, halogénure, aldéhyde, cétone, amine, acryl-amide, thiol, azoture d'acyle, halogénure d'acyle, hydrazine, hydroxylamine, halogénure d'alkyle, imidazole, pyridine, phénol, sulfonate d'alkyle, halogénotriazine, imidoester, maléimide, hydrazide, hydroxy, et azidoaryle photoréactifs.

17. Complexe de métal de transition selon la revendication 15, dans lequel au moins un des R_1 , R_2 et R'_1 est couplé à un squelette polymère.

18. Utilisation d'un complexe de métal de transition de formule



dans laquelle

M est le fer, le cobalt, le ruthénium, l'osmium, ou le vanadium ;

L est un ligand bidentate comprenant au moins un cycle imidazole ;

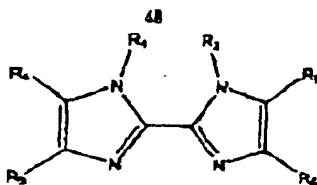
c est un nombre entier de -1 à -5 ou de +1 à +5 indiquant une charge positive ou négative ;

X représente au moins un contre-ion ;

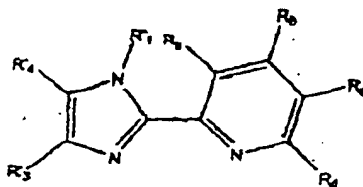
d est un nombre entier de 1 à 5 représentant le nombre de contre-ions, X ; et

L_1 , L_2 , L_3 et L_4 sont des ligands, à titre de médiateur redox.

19. Utilisation selon la revendication 18, dans laquelle L est choisi dans le groupe composé de :



et



où et

R_1 , R_2 , et R'_1 sont, indépendamment, -H ou des groupe alkyle, alcényle, ou aryle substitués ou non ;

R_3 , R_4 , R_5 , R_6 , R'_3 , R'_4 , R_a , R_b , R_c et R_d sont, indépendamment, -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂, ou des groupes alcoxy-carbonyle, alkylaminocarbonyle, dialkylaminocarbonyle, alcoxy, alkylamino, dialkylamino, alcanoylamino, aryl-carboxamido, hydrazino, alkylhydrazino, hydroxylamino, alcoxyamino, alkylthio, alcényle, aryle, ou alkyle substitués ou non.

20. Utilisation selon la revendication 18, dans laquelle L_1 comprend un composé hétérocyclique contenant au moins un atome d'azote.

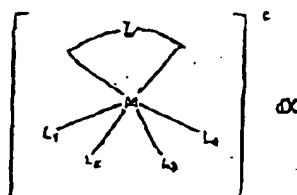
21. Utilisation selon la revendication 18, dans laquelle un ou plusieurs des L , L_1 , L_2 , L_3 et L_4 comprend (ou comprennent) un composé hétérocyclique couplé à un squelette polymère.

22. Utilisation selon la revendication 18, dans laquelle au moins un des L , L_1 , L_2 , L_3 et L_4 est couplé à un squelette polymère.

23. Polymère comprenant :

un squelette polymère ; et

un complexe de métal de transition répondant à la formule suivante :



dans laquelle

M est le fer, le cobalt, le ruthénium, l'osmium, ou le vanadium ;

L est un ligand bidentate comprenant au moins un cycle imidazole ;

c est un nombre entier de -1 à -5 ou de +1 à +5 indiquant une charge positive ou négative ;

X représente au moins un contre-ion ;

d est un nombre entier de 1 à 5 représentant le nombre de contre-ions, X ; et

dans lequel au moins un des L , L_1 , L_2 , L_3 et L_4 est couplé au squelette polymère.

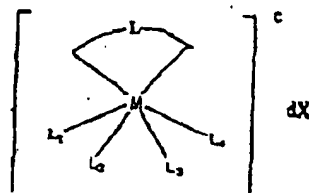
24. Polymère selon la revendication 23, dans lequel le squelette de polymère est choisi dans le groupe composé d'un copolymère de styrène/anhydride maléique, d'un copolymère d'éther méthylvinyle/anhydride maléique, d'un copolymère de polychlorure de 4-vinylbenzyle, d'un copolymère de polyallylamine, d'un copolymère de poly 4 vinylpyridine, d'une poly-4-vinyl-pyridine, d'un poly-N-vinylimidazole, et d'un poly-4-styrènesulfonate.

25. Détecteur comprenant :

une électrode de travail ;

une contre-électrode ; et

un médiateur redox disposé à proximité de l'électrode de travail, le médiateur redox répondant à la formule :



dans laquelle

EP 1 230 249 B1

M est le fer, le cobalt, le ruthénium, l'osmium, ou le vanadium ;

L est un ligand bidentate comprenant au moins un cycle imidazole ;

c est un nombre entier de -1 à -5 ou de +1 à +5 indiquant une charge positive ou négative ;

X représente au moins un contre-ion;

d est un nombre entier de 1 à 5 représentant le nombre de contre-ions, X ; et

L₁, L₃, L₂ et L₄ sont des ligands.

26. Détecteur selon la revendication 25, comprenant, en outre, une enzyme disposée à proximité de l'électrode de travail.

27. Détecteur selon la revendication 25, dans lequel au moins un des L, L₁, L₂, L₃ et L₄ est couplé à un squelette polymère.

28. Détecteur selon la revendication 25, dans lequel le médiateur redox est disposé, de manière non lixiviable, sur l'électrode de travail.